

Where to Go from Here

THE ubiquitous Mr Harvey Brooks and his committee of the OECD have made a valuable if preliminary attempt to describe how the now familiar machinery for the regulation of science policy may have to be adapted to meet the particular demands of the 1970s (see page 6). Rightly, the committee draws attention to the way in which the expectations of societies and the needs of governments have changed since science policy became a selfconsciously considered part of government policy in the late fifties. It would be unreasonable to complain that the outcome of this study consists either of somewhat general exhortations to those who make policy in national governments or specific proposals that the OECD itself should set up as an overseer, for this is essentially a field in which an accurate and sensitive description of a problem to be solved would in itself represent an important advance of understanding. It is also understandable, though perhaps less easily forgiven, that the committee's report should occasionally distract readers from the truth by making more than is strictly deserved of the OECD's previous contributions to the making of science policy. Is it really true that the OECD's "technological gap studies and their discussion at the Third Ministerial Meeting of Science proved to be a turning point in the evolution of science policy, in that they called attention to the problems posed by the nature of technological innovation"? It may be too much to ask that committees should bite the hand that set them up. In any case, the question is how to assess the committee's analysis of the changing pattern of demand on organized science and technology.

How real is the distinction which the committee makes between the three postwar phases of the "overall political climate in which science has operated"? The first period is said to be that until the early 1960s when electors and their governments (especially on the winning side) were persuaded that science and scientists were valuable and even prestigious. According to the committee, the second phase was one in which economists and systems analysts took an increasingly sceptical view of the place of science and technology in modern society and in particular of such problems as the best way of using science and technology to advance the cause of developing nations. According to this view of how postwar policies have evolved, the second still optimistic view of the potential contribution of science and technology came to an end in 1967 or thereabouts with the emergence of a period in which society at large questioned and is questioning the aims of science policy and even of science and technology themselves. The essence of the committee's argument is that governments have no alternative but to respond more adequately than in the past to the implicit demands which have been made on them and the services which they provide by an increasingly articulate and discontented population, and that, of course, is right. Moreover, many of the specific suggestions in the committee's report are sensible and stimulating. But there is a danger that the committee's description of postwar history may be too simple to be taken seriously.

The belief that the decades immediately after the war were times when science and scientists were uniformly

held in high regard and looked to for beneficent innovation of all kinds is certainly a disservice to many of those, professional scientists and others, who consistently in this phase sought to keep alive the old Wellsian view that science and technology are liberalizing influences which cannot be divorced from wider social considerations. In retrospect, the history of the years immediately after the Second World War is dominated not so much by overconfidence in the potential benefits of science and technology as by the concentration of scientific and technical effort on largely military problems. This, after all, was the period in which countries such as Britain learned with shock of the extent to which many industries were less productive than they might be for lack of deliberate policies for technical development and for lack of the men to implement them. This, as it happens, was also the period during which a great many governments made decisions since shown to be unfortunate about the potential uses of nuclear power and about the value of substantial investment in space research, but these and other developments were by no means universally acclaimed, even by governments or government agencies. There is perhaps more truth in the committee's observation that during the 1960s there was a tendency to regard investment in research and development as a desirable goal in its own right, even if it has always been plain that indices such as the proportion of a country's gross national product spent on research and development have never been more than useful yardsticks in the comparison of similar entities. The seeds of present anxiety about the social value of investment in research and development were, in other words, already plain in the debates which there have been in the decades since the Second World War about the possible hazards of nuclear power stations or the usefulness of space research. It would have been helpful, in other words, if the OECD committee had been able to emphasize not so much the discontinuity in the background against which science policy must be framed as the continuity of the themes which have been evident for half a century or more. In the nature of the political process, there is now a danger that too many governments will neglect the lessons that could be learned from the lessons of the recent past and that they will at the same time respond too sharply to current anxieties such as the entirely well intended concern for the natural environment.

What is the place of science and technology in the making of government policy at present and in the years ahead? So far as it can be expected to go, the OECD committee is luckily on the side of the angels. First, it acknowledges that there is bound to be a continuing connexion between research or technical development and communal prosperity, and that even advanced nations are not yet so well-to-do that they can snap their fingers at the prospect of being better off. That is a valuable principle to have repeated. The difficulty to which the committee points is that industrial expansion and economic prosperity do not follow automatically from successful research and development. Management and money are necessary if economic benefits are to be wrung



from what the technical people do, and there is a danger that the committee's valuable emphasis on the importance of the applications of science and technology to the service industries will be as frustrating as recent experience in industry if this lesson is not fully appreciated. Moreover, it is often exceedingly hard to tell in quantitative language just what will be the benefits of technical improvements in the services which form the framework of modern society. What, after all, is the economic benefit of the telephone system in a country such as Britain? Presumably, the annual cost of operating the telephone system, roughly £500 million, is a lower limit. Properly to quantify the value of the system would, however, be a much more serious undertaking requiring all kinds of studies not so far attempted. So many are the difficulties in this and similar problems that the laudable demand of the OECD committee that they should now be tackled seriously is to be interpreted not so much as a change of direction as an appreciation that the techniques of applied science and technology have now become sophisticated enough to be applied even to problems which are more complicated and less tangible than those considered (unwisely perhaps) to be fit subjects for study immediately after the Second World War.

The second issue raised by the committee's report is that of deciding to which objectives resources of a more sensitive and socially oriented technical effort should be directed. Should effort be spent on the SST or on the development of better health services? So far as its report goes, the committee is right to say that the setting of priorities must be determined by an assessment of costs and benefits which goes beyond the mere counting of numbers. In other words, it is right and proper that governments should occasionally determine their policies by considerations which are not always objective—if an electorate wants cleaner air (and is prepared to pay for it), for example, a government is as bound to respond to such an interest as to a demand for a better health service. In other words, at some level the decision as to how priorities should be determined in the deployment of scientific and technical research has become political. This, of course, is as it should be. For many governments, the practical question will be to work out ways in which to make intrinsically political judgments about matters which are often so technically obscure that open discussion is almost

impossible. The OECD committee has dipped its toe into this ocean by pleading for more and better cost-benefit analysis. It has not seriously tackled the question of how to give electors a sense of having helped to determine the objectives for research and development without encouraging fickle alternations of policy. An important part of the committee's report is concerned with the relationship between higher education and the setting of priorities in research. One of the lessons of the past two decades has been that no amount of wishful thinking can make trained people appear by magic in response to some new need. The committee is right to draw attention to the importance of patterns for higher education that will yield people who are more flexible.

How is all this to be administered? Here again the OECD committee has made a sensible start on an exceedingly difficult problem. The notion that there should be a blend in the structure of government machinery of central organizations concerned with research and development as a whole and more particular organizations concerned with potential applications of science is sensible as far as it goes, and a simple reflexion of the ways in which most governments at present function. That said, could not the OECD committee have gone at least as far as to urge that machinery for supporting research at institutions for higher education should not be isolated (as it is in the United States) from such machinery as there may be for supporting higher education as a whole? Should there not be deliberate links between defence research and the civilian departments of government that might be able to exploit defence research for civilian purposes? And while there may well be some benefit in making sure that some parts of the government machine should be set up so as to make assessments of the consequences of government policy (in which connexion the British Cabinet Policy Review Group under Lord Rothschild is held out as a good example), none of this solves the problem of how to provide ordinary voters with a sense of having been properly consulted about matters which they sometimes fail to understand. In short, valuable though the OECD's report may be as a stimulus to further thought on several important issues, there is probably a great deal to be done before the governments of advanced nations will learn not merely how to exploit science and technology but, more important, how to do it.

Race, Intelligence and Professor Eysenck

DR H. J. EYSENCK, professor of psychology at the Institute of Psychiatry at the Maudsley Hospital in London, who was once described as the Herman Kahn of psychoanalysis, has now called down a fresh round of thunderbolts with a book *Race, Intelligence and Education* (Maurice Temple Smith Ltd, £0.70). Professor Eysenck's objective is to discuss and to defend the argument put forward two years ago by Dr A. R. Jensen that measured differences of IQ between black and white children in the United States are very probably inherited. Nobody should complain that either Dr Jensen or Professor Eysenck has chosen to take up the question of the inheritability of IQ, even in the potentially explosive context of differences between black and white children (see *Nature*, 228, 1013; 1970). Indeed, both of them are right in saying that if

the problem is tractable at all, society as a whole would benefit enormously from a clearer understanding of the origins of variability in human populations. One consequence of such enlightenment, for example, would be a better understanding of how the educative process might be suited to the needs of individual children. The difficulty is that, true to form, Professor Eysenck has chosen to present a supposedly scientific argument without the qualifications to which any audience, but especially a lay audience, is entitled and with such a disregard for the way in which his sentences might be misquoted that his book may have the opposite effect from his expressed objective of helping to throw light on an important social problem.

To be fair all round, it is therefore important that Professor Eysenck says explicitly in his book that "those

who still continue to deny the black man equality and justice bear a heavy burden of guilt". In exactly the same spirit, Professor Jensen has said, "I do not advocate abandoning efforts to improve the education of the disadvantaged—I urge increased emphasis on these efforts . . .". Both statements are clearly intended not as disclaimers of wider responsibility but, rather, as honest expressions of intention, so that neither of them can be criticized for malevolence.

The issue is much more subtle, and, in one sense at least, is bound up with the question of how scientific hypotheses should be formulated, tested and, where appropriate, translated into social policy. One starting point for discussion, that advocated by those described as environmentalists in Professor Eysenck's book, is the null hypothesis that there is no difference between the intelligence of the black and white races which coexist in the United States that cannot be explained by differences in the ways in which the two groups are conceived, gestated, born and brought up. To be sure, there are problems about the definition of intelligence, and while IQ seems in general to be well correlated with what most people intend to imply by the concept of intelligence, there is always the possibility that in the comparison between groups which are intrinsically diverse, IQ may be very little better than a measurement of IQ. Similarly, there are serious doubts about the meaning to be attached to differences in such a context. What is significant and what insignificant? For the environmentalists, of course, there is always the awkward likelihood that intelligence in some shape or form has been a conspicuous element in the process of natural selection which has provided human beings with few advantages except intelligence to survive in competition with, say, lions. The trouble, unfortunately, for Professor Eysenck and for public understanding, is that it is no more sensible to discard the null hypothesis as a guide to action in some important field simply because there are clear exceptions to the general rule than it is to discard Newtonian mechanics in the consideration of how billiard balls move simply because there are circumstances in which sufficiently fast particles must be treated relativistically.

Professor Eysenck's argument is, as it turns out, not so much a denial of the null hypothesis as an attempt to marshal all the evidence in favour of the hypothesis that intelligence is 80 per cent inherited and 20 per cent environmental in origin. To this end, he describes the evidence which now exists in enormous quantities in the United States and elsewhere which reveals differences in several measures of intelligence between groups of white and black people. He is also able to use psychological studies of inheritance of attributes related to intelligence within more homogenous groups—identical twins who have been separated, for example—but no amount of detailed discussion can by itself demonstrate conclusively that the observed frequencies are indeed genetic. It is no triviality to complain, for example, that there is nothing in Professor Eysenck's argument to show that differences of diet cannot account for differences of performance in IQ tests. In circumstances like these, is it fair of him to tell the world at large that he has arrived "patiently and with scientific impartiality at the conclusion that the white race is inherently more intelligent [and the word is frequently used as a substitute for IQ] than the black".

A more serious, if less tangible, flaw in Professor

Eysenck's argument is that he fails to show how the apparently static differences between different population groups might become less conspicuous in a context in which more account was taken of the change of measured intelligence or IQ in different circumstances. To be sure, Professor Eysenck does acknowledge that severe deprivation in early childhood can seriously retard the development of IQ. He does not, however, take sufficient account of differences such as that between rural and urban populations. The argument that white children in the United States perform better in IQ tests because the tests themselves have usually been designed by white psychologists is easily rejected, as Professor Eysenck says, but is it possible to reject as lightly as he does the argument that IQ as such may be a more inappropriate measure for some populations than for others? After all, the development of IQ testing was not originally intended as an end in itself but rather as a means of selecting children for different kinds of education. Events have shown that for that purpose, IQ is too sensitive a measure and that some more gross comparison matches more fittingly with the uncertainty inherent in the educational system. In circumstances like these, it would be more appropriate if the comparison between different racial or social groups within society at large were also conducted with the help of such a scale.

100 Years Ago



THE very ingenious manner in which Mr. Howorth first misrepresents Darwinism, and then uses an argument which is not even founded on his own misrepresentation, but on a quite distinct fallacy, may puzzle some of your readers. I therefore ask space for a few lines of criticism.

Mr. Howorth first "takes it" that the struggle for existence "means, in five words, the persistence of the stronger." This is a pure misrepresentation. Darwin says nothing of the kind. "Strength" is only one out of the many and varied powers and faculties that lead to success in the battle for life. Minute size, obscure colours, swiftness, armour, cunning, prolificness, nauseousness, or bad odour, have any one of them as much right to be put forward as the "survival of the fittest." The error is so gross that it seems wonderful that any reader of Darwin could have made it, or, having made it, could put it forward deliberately as a fair foundation for a criticism. He says, moreover, that the theory of Natural Selection "has been expressively epitomised" as "the persistence of the stronger," "the survival of the stronger." By whom? I should like to know. I never saw the terms so applied in print by any Darwinian. The most curious and even ludicrous thing, however, is that, having thus laid down his premises, Mr. Howorth makes no more use of them, but runs off to something quite different, namely, that *fatness* is prejudicial to fertility. "Fat hens won't lay," "overgrown melons have few seeds," "overfed men have small families,"—these are the *facts* by which he seeks to prove that the *strongest* will not survive and leave offspring! But what does nature tell us? That the strongest and most vigorous plants *do* produce the most flowers and seed, not the weak and sickly. That the strongest and most healthy and best fed wild animals *do* propagate more rapidly than the starved and sickly. That the strong and thoroughly well-fed backwoodsmen of America increase more rapidly than any half-starved race of Indians upon earth. No *fact*, therefore, has been adduced to show that even "the persistence of the stronger" is not true; although, if this had been done, it would not touch Natural Selection, which is the "survival of the fittest."

ALFRED R. WALLACE

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OLD WORLD

NUCLEAR PHYSICS

Cutting Corners

BOTH the Rutherford Laboratory and the Daresbury Nuclear Physics Laboratory seem to be coming successfully to terms with reductions in their budgets for 1971-72. The Rutherford Laboratory has been allocated only £7.4 million (£600,000 less than in 1970-71 and more than 10 per cent less in real terms) and Daresbury is faced with a budget reduction from £4.4 million to £3.7 million.

These economies have been fully anticipated and stem from the decision of the United Kingdom to participate in the CERN 300 GeV accelerator project at CERN. The two national accelerator laboratories between them account for more than 20 per cent of the total Science Research Council budget and about half the money spent on nuclear physics.

The Nuclear Physics Board of the SRC is also considering the long-term future of nuclear physics research in Britain and plans for Daresbury and Rutherford during the next five or ten years will inevitably be dictated by the performance of the laboratories in the somewhat straitened circumstances of the next year or so. A spokesman for the Rutherford Laboratory said this week that if the laboratory is viable on a smaller budget, there will be no reason to fear more drastic cuts or even closure. Reductions of expenditure at the Rutherford Laboratory are being made right across the board, however, which means that less high energy physics will be carried out in future. But any move to make especially swingeing cuts in the applied research programme (for example, the development of superconducting magnets) in order to maintain a high level of high energy physics research would be self-defeating in the long run.

Professor A. Ashmore, Director of the Daresbury Laboratory, says that he will try to buy as little new equipment as possible. But at least one of the dreams at Daresbury, the proposal for a 20 GeV electron synchrotron, now seems unlikely to materialize in the near future. The idea that Daresbury should house a £3.5 million national nuclear structure facility, probably centred on a Van de Graaff generator producing a voltage of at least 20 MV seems, however, still to be alive although a formal proposal to the SRC is unlikely to be made for at least a year. Nevertheless, the prospects seem good because finance for the design study was made available at the end of last year, and the design team is already at work at Daresbury.

CONTRACT RESEARCH

Fulmer Shows the Way

CONTRACT research in the United Kingdom can be a lucrative business if it is properly managed. That is the conclusion to be drawn from the report for 1970 of the Fulmer Research Institute. The report shows that turnover increased by 20 per cent to £360,000 and that profits soared to £25,500, even after a contribution of £9,100 to Fulmer's owners, the Institute of Physics. But contract research by independent organizations in the UK is still small beer compared with the United States where the Battelle Institute and Stanford Research each have an annual turnover of about £50 million and the total market is worth about £250 million a year.

The contract research business in the US profited in the years since the Second World War from the US government's policies of placing research contracts with independent organizations, but this source now seems to be fast drying up and the volume of business has diminished during the past two years. In the UK, on the other hand, the government has never encouraged contract research to the same extent and the commercial organizations have had to compete with government-aided research associations, for example, for research farmed out by industrial companies. But absolute discretion seems to be the factor which attracts some companies to the independent organizations. Contract research in the UK is now worth £5.6 million a year—more than twice the figure for 1965—and is chiefly carried out by eight companies, the largest of which, the Huntingdon Research Centre, has an annual turnover of £2 million.

Independent Contract Research Organizations in the UK

	Turnover (£thousands)
Huntingdon Research Centre	2,000
International Research and Development	1,100
Robertson Research International	740
Ricardo and Co.	740
Fulmer Research Institute	360
Inveresk Research International	250
Yarsley Research Laboratories	230
Cambridge Consultants	200

The Fulmer Research Institute employs a staff of 137 of whom 52 are graduates. Contracts from the UK government have always accounted for a substantial part of the institute's income but between 1960 and 1970 this

proportion has dwindled to less than half the overall total, partly because of the rapid growth of contract research facilities at Harwell. Of the work undertaken for industry, 60 per cent comes from the UK and the remainder from overseas.

Most of the research work at Fulmer in its early days was metallurgical, and although this expertise has been expanded, the past few years have seen a growing interest in other materials such as plastics, polymers, ceramics and glasses, and an increase in technical services—electron microscopy, X-ray diffraction and so on—to attract customers. The Fulmer Research Institute has also been quick to realize that traditional contract research is necessarily limited by the prevailing economic climate and during the past year or so money has been earmarked for research projects initiated within the institute rather than by an outside sponsor. The idea is that industrial support will be sought for the institute's own patents and the royalty income used to expand these activities further.

RABIES

Quarantine Upheld

THE Committee of Inquiry on Rabies, set up early in 1970 after the deaths in Britain of three rabid dogs, made its final report this week. Britain has been virtually free from rabies for almost fifty years, and the committee now urges that the quarantine regulations should be reinforced, not relaxed. The committee takes the line that a lapse of vigilance might produce an epizootic of rabies in Britain comparable with that on the European mainland.

The report proposes that all categories of mammals entering Britain, with certain exotic exceptions, should be subject to strict quarantine controls for six months. The exceptions which, for ecological or physiological reasons, are unlikely to harbour rabies virus include seals, elephants, dugongs and aardvarks. It seems likely, the report says, that all mammals are susceptible to rabies, and that therefore "no exemptions to general quarantine requirements should be permitted".

The report argues that vaccination without quarantine would not prevent the introduction of the disease into Britain. The committee is clearly impressed with the view that a single rabid animal which escaped the net could start a rabies epizootic in Britain.

Vaccines in current use are of two kinds, live or inactivated. Live virus vaccine produces a high degree of immunity which lasts for a long time, but may cause encephalitis in animals for which it was not intended and can give rise to false results in laboratory

tests for rabies. Inactivated vaccines, on the other hand, are not as effective in promoting immunity but are otherwise free from these complications.

Rabies vaccine for veterinary purposes is distributed in Britain by May and Baker Ltd, which obtains its supplies of the vaccine 'Ribaffa' from France. This is the vaccine which was used during the rabies scare which led to the committee of inquiry. Rabies vaccine for human use in Britain is distributed by the government's public health laboratories, which obtain their supplies from E. I. Lilly, the American pharmaceutical company.

However sensible the recommendations of the committee may seem to be, its report will win it no friends among those of the nation's pet-owners who pressure the quarantine regulations. The committee says that "we have reason to know that dog-owners are not over-scrupulous where possession of their dogs is concerned. . . . The greatest possible vigilance is therefore needed against the illegal importation, by whatever means, of potentially infected animals".

SCIENCE ON STAMPS

More Than Face Value

THE science advisers to the Nicaraguan Post Office clearly know more science than they could write on the back of a postage stamp, but they have commemorated the chief theoretical breakthroughs of two millennia of scientific progress in just this way. The stamp illustrated below, which shows Pythagoras's equation against a background of its applications in geometry and architecture, is one of a set of ten that the Nicaraguan Government has issued to highlight "the ten mathematical equations that changed the face of the Earth". The face of each stamp illustrates an equation, and there is an explanatory paragraph on the back.



Few will jib at the choice of equations; at 10 centavos (rather less than one new penny) there is $1+1=2$, the basis of counting, while 15 centavos buys Newton's Law of Gravity. Einstein's $E=mc^2$ rates 20 centavos, while de Broglie's equation, essential groundwork for the Schrödinger equation,

adorns a 1 cordoba (about 6 new pence) airmail stamp. Top of the price scale are Napier on logarithms and the Maxwell equation, each of which rates a 2 cordoba airmail stamp. Boyle's Law, Faraday's electrochemical equivalent, Einstein's equation for stimulated emission, Planck and Bohr are among those which will no doubt be celebrated in the next issue.

RESEARCH FELLOWSHIPS

Lindemann Bequest

THE English Speaking Union announced details last week of a new research fellowship scheme designed to encourage young British scientists to further their careers through a year or so spent in the United States. The scheme has been made possible through the generosity of the late Brigadier C. L. Lindemann, brother of Lord Cherwell, who was scientific adviser to Churchill during the Second World War.

In his will, Brigadier Lindemann assigned funds to be used to enable exceptionally talented graduates to continue pure research in the physical sciences in the United States. Recommendations for fellowships will be made by a committee chaired by Lord Sherfield and whose ranks include Sir Patrick Dean, Dr M. A. Grace, Professor A. R. J. P. Ubbelohde, Professor C. A. Coulson, Professor R. V. Jones, Professor Kingsley Dunham, Professor F. Hoyle and Professor D. H. R. Barton.

The fellowships are open to citizens of the British Commonwealth who are under 30 years old and who graduated from a UK university. The stipend will be generous—around \$10,000 a year—which should ensure no shortage of applicants. By way of further inducement, the successful candidate's return fare to the US will be paid, together with some part of the cost of moving his or her family.

BFMIRA

Rehabilitation of an RA

THE British Food Manufacturing Industries Research Association, which only five years ago seemed certain to succumb to financial and administrative difficulties (see *Nature*, 211, 561; 1966), looks now to be well on the way to restoring its reputation and fortune. For the past three years the association has shown a surplus on its income and expenditure account; in 1970 the balance was £24,763 compared with about £14,000 in 1967 and a deficit of £16,000 in 1966.

The most heartening sign of BFMIRA's recovery, however, is the proportion of its income now derived from services to its member industries. Income from these activities, which include analytical, microbiological and information services, showed an increase of almost 60 per cent over the 1969 total to top £27,000. The value of confidential projects tackled on behalf of member industries more than doubled in 1970 to bring in £25,000, a sure sign that the association is beginning to live down its troubled past, and that confidence in the discretion and quality of the research undertaken by the association is returning.

Like all RAs, BFMIRA is partly supported by the government; its grant is valued at 45 per cent of whatever the association can raise from industry in subscriptions. Last year the government subsidy was £58,853 on a subscription income of £132,737. This latter figure has remained static for three years now, and seems unlikely to increase much in the future, for the association has set its face against increasing the cost of subscription except for the inevitable cost of living adjustments. Nevertheless, the association is within striking distance of the maximum amount—£80,000—that the government will grant. On special projects, such as meat biochemistry, the government meets every pound from industry with a pound of its own, and here again BFMIRA is within a few thousand of the ceiling.

Despite these signs of viability and a turnover of about £336,000, BFMIRA is still a long way from being self-sufficient and cannot compete with the giant Huntingdon Research Centre, the largest commercial biological testing centre in Britain with a turnover in 1970 of about £2 million. To match Huntingdon's success is clearly first priority in the eyes of the BFMIRA management, and they feel that some of the cards are stacked in their favour. The association is non-profit making, nor is it liable for Corporation Tax. Furthermore, the association lies between the extremes of Huntingdon, which has to charge a commercial price for its services, and the government financed Food Research Institute at Norwich, which, the customers that BFMIRA is trying to attract feel, has a far too academic approach to their problems.

This should ensure some measure of success for BFMIRA in the future, but clearly old wounds are hard to heal; in setting out ambitious plans for the association, the editorial in a recent *BFMIRA Bulletin* (June 1971) wryly observes that should its plans go astray the association may well be "the laughing stock of the industry", a strangely diffident note amidst otherwise hopeful proposals.

Helmsmen Peer Ahead

A BRAVE and mercifully succinct account of how science and technology may be more closely geared to social, as distinct from economic, progress has now been published by an *ad hoc* committee of the OECD under Dr Harvey Brooks, dean of engineering at Harvard University. The original intention was that the committee should attempt to identify current trends in the making of science policy and to suggest how these would influence not merely the institutions which are directly concerned but also society at large. In practice, the committee has done as much as any contemporary organization of comparable weight to show how the goals of science and technology can be made credible in the world of the Domsday men.

The committee says that in the seventies it will be plain to the governments of developed nations that economic growth as such "is no longer a sufficient overall objective" and that it will be necessary, in the planning of industrial policy, to make "further interventions in the working of the market economy". What the committee implies is that in spite of past successes and future undeniable promises, science and technology must increasingly be judged by the quality of their contribution to society as well as by the degree to which they contribute towards prosperity.

On economic growth, for example, the committee says that although most OECD governments expect that economic growth in the 1970s will be comparable with that in the past decade, "as growth is taken more for granted" inflationary pressures will be increased by the greater competition of interested groups for a larger share of each nation's GNP. At the same time, there will be a need to invest in what is called "social infrastructure" and to demand that societies should be organized in such a way as to be free of internal conflicts. The committee acknowledges that one result may be the risk that developed nations will regard economic growth and the science and technology on which it depends as "sources of disharmony" but that the seventies will be suffused with an unwillingness of the general public to accept that the objective of harmony between people and their environment will necessarily be a costly process.

In the circumstances, the committee comes out staunchly for science and technology as harbingers of constructive and indeed welcome change, but it acknowledges that governments will increasingly seek to influence the way in

which industrial output is composed of ingredients with differing social benefit, that the international character of the impact of science and technology will be increasingly preoccupying, with the result that "the management and protection of the global environment will require restraints on national sovereignty for which there is very little precedent"; that there is a danger that contrasts between developed and underdeveloped nations will mean that the "inappropriate technology of advanced nations" is thrust upon the rest of the world; that "gaps" between rich and poor countries will be at once a challenge and an impediment to the exploitation of science and technology; that international corporations will be a constant problem and that these issues will colour the making of science policy in general.

The report of the committee attributes the "difficult and sometimes painful" adjustments of the scientific establishment to new priorities as consequences of uncertainty about and rapid change of social objectives. In the circumstances, it says, there are bound to be serious difficulties in forecasting needs of scientific manpower in particular fields. The committee also says that it is wrong to blame the "deleterious effects of technology" on the choice of priorities in research and development, for these are in fact simply consequences of social and economic policies. At the same time, the committee guesses that "public disillusion" with the effect of technology on the quality of life, at present evident in "outcries" about urban decay, pollution and redundancies, is likely to increase. And what, in any case, is the "ethical basis of research whose results might be used for the manipulation of the individual or society"? The committee's view is that many present discontents stem from the insufficient application of technology to service industries as distinct from manufacturing, that economic growth will in any case be necessary in advanced countries so as to remove "residual islands of poverty" as well as to provide the resources for urgent social development but that advanced nations would increasingly be concerned with the value of economic growth as a means of generating social development rather than increasing personal incomes. In the circumstances, there is "an overwhelming need to reassess" the place of science and its component parts in cultural terms as well as from the point of view of potential productivity.

On the management of science and

technology, the committee says, that the most perplexing problem is that of deciding between centralization for science and technology and the management of scientific and technical matters as a part of general social and economic policy. The contrast is that between the centralized policies which have recently been adopted by several European nations and the sectorial policy traditional in the United States which is, the committee says, less satisfactory when social goals are changing rapidly. One of the deficiencies of the sectorial approach, the committee says, is that there is a danger that potentially important applications of science and technology to the needs of developing countries will be neglected (but there is a proper warning that it is not easy to transfer technology in this way).

In the circumstances, the committee considers that the OECD may have an important role to play in the evolution of modern science policy because of its existing international connexions. The starting point for this view is that science and technology should be considered integral parts of social and economic policy-making. The committee wishes to see more studies by the OECD of matters such as the process of innovation as well as the operation of the money system, the fiscal system, the incomes policy and the labour market in a technological society. There needs also to be on a national as well as an international basis a closer study of the relationship between technical development and the disparities of prosperity, the mobility of labour and the process of inflation.

The committee asks that governments should deliberately encourage socially valuable technology with the neglect, if necessary, of less desirable work and that there should in particular be an attempt to improve productivity in the public services. OECD itself should become an authority on the problem of how to assess the risks and benefits of particular technical developments and, the committee says, the organization should be prepared to spend money on work of this kind.

In general, the committee considers that education policies usually neglect the differences in the demand for education of particular kinds and the capacity of institutions of education to meet those demands, and goes on to ask that the future development of higher education should be designed so as to provide a more flexible basis for professional work. The committee says that higher education should be "democratized" in the sense of more equal

opportunities for all candidates.

On fundamental research, which the committee regards as "an essential element in the total science and technology system", the committee asks that advanced nations should acknowledge their obligation to provide help for the advancement of fundamental research and that support for basic research should not be diminished in the search for new priorities in the application of science and technology. At the same time, in spite of the advantages of some degree of competition between nations in science, the committee considers that the countries of the OECD at least should be "moving away" from national competition towards closer international collaboration, especially where conspicuous national facilities are concerned.

On the view that most nations will in future require a blend of centralized and sectorial policies for science, the committee argues that the OECD should carry out studies that would inform the making of policy for science and technology in fields such as mass transportation, land use, the postal services and solid waste disposal. Nevertheless, the committee is anxious that no nation should so commit its resources in science and technology to specific fields of interest that money cannot be spared for long-term and basic research. The committee follows a cautious line on the environment but it does ask that individual nations should not act unilaterally in the imposition of standards without calculating the consequences for the pattern of international trade. This, no doubt, is an oblique reference to such things as the determination of the United States Congress to impose restrictions on the effluents from motor car engines.

The committee also asks that the OECD should take a lead in persuading governments to set up suitable machinery for the formulation of science policy. Readers of the report will, however, be at a loss to know whether the OECD is asking for a ministry of science in every member nation by urging the "creation of a governmental mechanism, operating under specific authorization and full support of the head of government, with responsibility for investigation of long-range policy issues". An important part of its argument is that a great deal of the planning of science and technology cuts across the lines on which government departments are at present organized, and that there should be formal machinery for reflecting this pattern. At the same time, the committee takes the view that the OECD in general and national governments in particular should create special machinery for providing technical assistance to developing countries.

WEITZMANN INSTITUTE

Science and Society

from our Special Correspondent

Brussels, June 29

"We have had the extraordinary experience of having nine of the most experienced scientists for two days on a psychiatric couch." That is how Professor Albert Sabin, President of the Weitzmann Institute of Science, summed up a conference on the impact of science on society, held this week at the Palais de Congress in Brussels. Unfortunately, however, his metaphor was given an unintended meaning by a story related earlier at the conference. It was alleged that the famous mathematician Norbert Wiener spent three days on a psychiatrist's couch relating his problems, at the end of which the psychiatrist confessed his inability to make a diagnosis and Wiener left, none the wiser about his condition. To be sure, the conference lasted for only two days, but an experienced onlooker would probably have found himself in the same unfortunate position as Wiener's psychiatrist.

In spite of the considerable array of talent assembled at the conference (which was arranged by the European Committee of the Weitzmann Institute, and which consisted of about two hundred scientists, including five Nobel Laureates, government planners and industrialists), Sabin himself suggested at the end of the first day's session that the discussion was concerned too much with generalities and not enough with specific issues. Heeding this suggestion, therefore, Professor David Samuel, a biologist engaged on brain research at the Weitzmann Institute in Rehovot, took the discussion of the impact of science on society into the realms of the ethics of brain research. Although he freely admitted that his conclusions and suggestions are not novel, he provided a stimulating discussion of the possibilities open to the scientific community for regulating a specific area of research that has aroused great fears among laymen and also among some scientists. He put forward the suggestion that trends in brain research should be evaluated by the best people concerned, and that a code of ethics should be drawn up to cover the use of human volunteers, the permissible extent of drug trials and the moral aspects of some types of transplants. Such a code would be "not unlike the Hippocratic oath, and it would help guide scientists and show the public that guidelines are being laid down to guard the sanctity of people's minds".

Samuel was, nevertheless, sceptical about most of the horror stories perpetrated by uninformed commentators on the likely possibilities opened up by brain research. He suggested that the

idea that electrical stimulation of the brain can be used to change personalities, for example, is not a realistic proposition. Likening the human mind to a telephone system that is a million times more complex than the world telecommunications network, he said that "the complexity of the human mind is a sufficient safeguard against manipulation by a madman or a dictator". Language, culture, personal identity and political ideology are not controllable, he said, but on the other hand, brain research if correctly applied could lead to immensely valuable results.

Some participants did not, however share Samuel's optimism, Professor Aharon Katzir-Katchalsky among them. He told the conference that "there is no question that the public opinion that there is danger in this research has a lot behind it", and he questioned Samuel's suggestion that a value-system for regulating scientific research can come from science alone. But another popular suggestion that from time to time came under fleeting discussion—that a moratorium could be placed on some areas of scientific research—found few supporters. Katchalsky, for example, said, "Nobody knows how to stop research and the truth cannot be kept under control."

The burden of Katchalsky's message to the conference on the first day was that molecular biologists are facing a crisis of conscience equivalent to that faced by nuclear physicists after the atomic bomb—but at least they have the chance to think out the likely consequences of their research before it is too late. Treating the conference to a short survey of the more popular fears and moral problems faced by molecular biologists, he concluded that science has brought about the necessity for coming to grips with international politics, and that scientists should collaborate with people involved in public affairs to bring scientific methods to bear on social problems. But first they must put their own house in order.

In the event, what emerged from the conference was the impression, suggested by Professor Victor Weisskopf in the closing session, that "science is on the defensive". The ten speakers, including Sabin himself, gave the benefit of their 550 years of combined experience to the viewpoint that science, if properly used, is not necessarily an object to be feared or mistrusted. And, as Professor Jean-Jacques Salomon pointed out, "the simple fact that you are met here to discuss the impact of science on society proves no doubt that there is no cause for despair". Nevertheless, many of the participants vented the opinion during the coffee breaks that a large congress discussing general questions is not necessarily the best forum for stimulating debate.

NEW WORLD

Furtive Search for Cancer Head

by our Washington Correspondent

THE executive talent scouts at the White House are combing the land for a politically acceptable name to put at the head of the Cancer Cure Programme, the National Cancer Bureau, or whatever fancy name the present National Cancer Institute may end up with. At least in financial terms, the new post will be one of the most influential in the field of biomedicine, since its holder will dispose of funds totalling some \$330 million in the present fiscal year with promises of still greater largesse to come.

One of the leading candidates at present is Albert Sabin, developer of polio vaccine and now director of the Weizmann Institute in Israel. Several others under consideration, including R. Lee Clark of the M. D. Anderson Hospital in Houston, are members of the panel of consultants on the Conquest of Cancer, a group of fund raisers and cancer specialists which set in train the movement for an expanded financing of cancer research. The panel's advocacy of an independent cancer agency outside the National Institutes of Health was adopted by Senator Edward Kennedy and at first opposed by the Nixon Administration which sought to buy off the Kennedy plan by requesting from Congress an extra \$100 million for cancer research.

Last month Kennedy's health subcommittee agreed on a compromise bill that is in essence the Kennedy plan for an independent agency. The degree of independence from the NIH, however, seems to have been left unclear in the bill, and may to a large extent depend on the man who is appointed director of the agency.

The Nixon team searching for a director apparently includes James Cavanagh, a member of the White House staff, Elliot L. Richardson, Secretary of the Department of Health, Education and Welfare, Jonathan Moore, a counsellor to Richardson, and Edward E. David, the President's science adviser. The search process seems to be being conducted in a close-handed way, with various interested parties being asked to suggest candidates but given no indication of how the search is progressing. If true to Administration form, the successful candidate will have to be a Republican or at worst a neutral Democrat. A major issue has been whether the director should be a scientist or a manager. The panel of consultants, whose individual views have been

listened to by the Administration, stressed the importance of applying management techniques to the solution of cancer, and a candidate who combined administrative skills with a scientific reputation, preferably in or near the cancer field, would be an obvious choice.

Early in the selection procedure, a list of about fifteen names was in circulation, some of them non-scientists and many drawn from the twenty-six-member panel of consultants. Panel members said to be under consideration have included Henry S. Kaplan, chairman of the radiology department at Stanford University, R. Lee Clark, head of the M. D. Anderson hospital in Houston, Benno C. Schmidt, an investment banker who chaired the panel, Joseph Burchenal, vice-president of the Sloan-Kettering Institute for Cancer Research, and Joshua Lederberg, Nobel laureate and Stanford geneticist.

Two months ago, when President Nixon announced in an attempt to fight off the Kennedy bill that the National Cancer Institute would be elevated into a bureau within the NIH, the Administration's choice for head of the bureau was Kaplan. Kaplan later withdrew his name and the search had to begin again, doubtless made harder by the obvious political pressures to which, under the prevailing arrangements, the director will be exposed. Sabin, who seems to be one of the leading candidates, has already had discussions with David, the President's science adviser, and with people at the National Cancer Institute.

But Sabin said from Brussels, where he was staying early this week, that he has not been offered the job and he would "have to wait and see" if the offer were made. Sabin, who is setting up a multi-disciplinary cancer centre at Rehovot, said he believes that basic, non-target research in the biomedical sciences is "fairly well funded" at present and that the new money made available for cancer should go into targeted research programmes, not least as a trial of what scientists can do to apply their skills to national problems. These views are much in line with David's utterances on the question. Sabin also believes that the cancer operation must remain intimately tied to the National Institutes of Health, especially with respect to the processing of grant applications.

Clark, another prominent candidate, is said to have the backing of the panel

of consultants and is known for his administrative as well as fund-raising adeptness. But although Clark has more support than other candidates, he also has more opposition, in part, it seems, through fear of the same qualities. Clark is a believer in the power of management techniques and advocates that a master plan for cancer should be drawn up using such tools as time phase analysis. Clark said last week that he believed the job called for both a scientist and an administrator.

Among the scientific community probably a majority would prefer the new cancer effort to be headed by a scientist of merit who is also knowledgeable about cancer—James D. Watson, director of the Cold Spring Harbor laboratory, is one name mentioned as an example. Others believe that the new director need only be a figurehead who goes down well with Congress but would leave the scientific operation of the institute alone. At one time there were plans to announce the choice for the appointment on July 1, the date on which the extra \$100 million requested by the Administration at an earlier stage in its skirmish with Kennedy becomes available for spending. It now seems that the appointment may be postponed until the Kennedy bill, now adopted by the Administration in essential outline, has passed further through the Congressional mill and the structure of the new agency is somewhat better defined.

MANPOWER

PhDs in Surplus

by our Washington Correspondent

FOR the past seven years, federal officials have ignored the warnings of outsiders that the educational system was churning out PhDs at such a rate that the supply would soon exceed the demand. The Office of Education, for example, not the brightest assembly of wit in the federal government, was predicting in 1964 that there would be a cumulative deficit of 125,000 PhDs by 1974. It has taken almost the actual appearance of jobless PhDs to convince the forecasters that they were wrong. The National Science Foundation has now come into line with a projection* that calculates a sizable surplus of doctoral scientists and engineers—between 18,000 and 66,000—by 1980.

Between 315,000 and 336,000 doctorate holders in science and engineering will be available to the US economy

**1969 and 1980 Science and Engineering Doctorate Supply and Utilization. US Government Printing Office, Washington DC 20402. 50 cents.*

Table 1 Bureau of Labor Statistics Estimates of Occupational Employment, 1968, and Projected Requirements, 1980, for College Graduates

Occupation	Estimated 1968 employment	Projected 1980 requirements	Per cent change	Supply estimated to be
Chemists	130,000	200,000	55.7	Significantly below requirements
Dentists	100,000	130,000	31.7	
Physicians	295,000	450,000	53.1	
Physicists	45,000	75,000	63.9	
Engineers	1,100,000	1,500,000	40.2	Slightly short of requirements
Geologists and geophysicists	30,000	36,000	20.6	
Mathematicians	70,000	110,000	60.5	Significantly above requirements
Life scientists	168,000	238,000	40.8	
Teachers, elementary and secondary	2,170,000	2,340,000	7.8	

in 1980, according to the NSF projection, but only 270,000 to 297,000 jobs. The surplus will be worst for engineers, among whom PhDs are likely to exceed by 40 per cent the number of jobs available ten years hence. Despite the present shadow of unemployment, however, it is predicted that scientists will enjoy a virtual balance between supply and demand in 1980. Doctorate surpluses in other fields are for life sciences about 9 per cent; for mathematics about 10 per cent; and for sociologists 20 per cent. These surpluses are calculated from comparison of the middle of the ranges for supply and utilization of doctorates. The NSF's figures differ in detail, though not in broad outline, from those prepared by the Bureau of Labor Statistics for all college graduates (see Table 1).

The chief difference between the NSF's new projections and those it issued two years ago is a change in the assumptions about doctorate enrolment. Two years ago, the Office of Education, on whose assumptions the NSF study was based, guessed that graduate enrolment would have risen by 99 per cent in 1980. The NSF now expects enrolment to rise by only 43 per cent. Smaller enrolment means a considerable reduction in the demand for graduate faculty, the chief source of employment of PhDs.

Why was the PhD surplus, present and emerging, not predicted earlier? In fact it was, notably by Allan M. Cartter, an economist who is now chancellor of New York University. The gist of Cartter's prediction is the demographic argument that a decline in the student body in the 1980s—a consequence of the reduced fertility rates of recent years—will reduce the size of required faculty.

Why was Cartter treated like Cassandra? "Many people on the Washington scene, particularly in the Office of Education and NSF, but also among the university presidents and deans, felt that such projections were

damaging to the national interest and would jeopardize federal funding for graduate education," Cartter said recently, arguing that it would be "much better to be objective and attempt to make the case for graduate support on its merits, rather than ride the scarcity myth another two or three years and then have the Congress react strongly once the apparent crisis was past. If the superb efforts that Phil Handler and the NSF board made in 1968 and 1969 to try and convince the Congress that graduate education should be funded for its own sake had been made five years earlier, I believe that our universities might not be in the same painful straits that they are now in."

NATIONAL SCIENCE FOUNDATION

More Mauling in House

by our Washington Correspondent

THE House of Representatives has continued to rough up the National Science Foundation with a report just issued from the House Appropriations committee. The committee has recommended that in the fiscal year beginning this month the budget of the NSF be set at \$582 million, or \$38 million less than the amount requested by the Administration.

The House's first interference with the NSF's affairs was a rearrangement of its budget by the Science and Astronautics committee, which authorizes upper limits to the funds the Appropriations committee may recommend. In an unexpectedly authoritarian gesture, the inspiration for which is said to have stemmed from the committee's staff, the committee slashed the ambitious programme known as RANN (Research Applied to National Needs), restored the monies which had been pared from the NSF's educational programmes and compartmentalized the NSF budget to prevent these changes being reversed (see *Nature*, 231, 8; 1971). The House Appropriations com-

mittee has now followed these policies, recommending the full amounts authorized for the educational and institutional support programmes but cutting even further the funds for the group of programmes that includes RANN. In its report issued last week the committee also announced a cut of more than 10 per cent in the \$257 million requested for the support of individual research projects.

The decisions of the Appropriations committee have yet to pass the House but the authorization bill prepared by the Science and Astronautics committee sailed through last month with hardly a word of serious debate, unless the following interventions can be counted as such:

Mr Schmitz (a Republican from California): There has not been any discussion here about the social sciences. All the sales pitch on this bill has been on its more truly scientific aspects. As a former junior college social science instructor, I am quite familiar with the pseudo-scientific nature of many of the subjects encompassed under the broad heading of social sciences . . . [Referring respectively to studies of community organization, violence and demography.] Does that mean more for Cesar Chavez? Does that mean another Kerner report? Does that mean vote for Paul Ehrlich? . . . I think not only Members of Congress but the people would be interested in finding out just what we are being drawn into under the umbrella of science.

Mr Gross (a Republican from Iowa): I do not care what colour of Mother Hubbard dress that is put over this so-called foundation . . . This money will be spent on such esoteric and dubious projects as further studies to make sure that previous studies in social organization have not been howling flops, resulting in "imperfectly tested ideas" that would be extremely harmful if put to use . . . Money would be used to find out how man has used his environment "around the world". That is where it is proposed to get into the \$2 billion a year spending on this business. More money will be spent to learn how people have adapted to urbanization. I suppose that will involve asking various people, "How do you like living in town?"

The Senate, on present form, has been kinder to the NSF. The subcommittee that handles the NSF's authorizations, chaired by Senator Edward Kennedy, last month recommended a budget of \$703.5 million for fiscal 1972, an excess of some \$83 million above the President's request which may be designed to give the Senate some bargaining power against the lower sums recommended by the House. For good measure, Kennedy's committee took the unusual step of recommending an authorization for next year as well; for fiscal 1973 the committee wants the NSF to receive \$900 million.

NEWS AND VIEWS

Are Warts Cell Clones?

WITH tumour virology becoming one of the most fashionable and best funded areas of biological research, it is ironic that the biology of the only indisputable human tumour virus, the human wart or papilloma virus, which was first detected more than half a century ago, remains almost completely obscure. Admittedly warts are benign tumours, but the chief reason why the papilloma viruses have for so long been neglected is more mundane than that; nobody has yet succeeded in finding a satisfactory tissue culture system which supports the replication of, or can be transformed by, papilloma viruses. As a result, although the structure and composition of papilloma virus particles have been determined, almost nothing is known of their intracellular biology. The contrast between the vast accumulation of information about simian virus 40 and polyoma virus and the ignorance of their close relatives the wart viruses could hardly be starker. And so amidst the interminable flow of reports of often redundant work on polyoma virus and SV40 it is a refreshing change to read Murray, Hobbs and Payne's description (see page 51 of this issue of *Nature*) of their fascinating experiments which strongly suggest that warts are clones of cells.

There are two, not necessarily mutually exclusive, ways in which a tumour of viral aetiology might develop. The tumour might arise as a result of the continuous division of a single cell transformed by the tumour virus, in which case the tumour would, of course, be a clone of cells. Alternatively, the first cell to be infected and transformed might liberate progeny virus particles, which, by infecting and transforming adjacent cells, would recruit them to the tumour. The RNA tumour viruses, which usually replicate in the cells which they transform, are potentially able to cause tumours in the second manner and indeed there is experimental evidence which indicates that foci of cells transformed *in vitro*, and tumours induced in mice by mouse sarcoma virus, develop at least in part by recruitment.

By contrast, polyoma virus and SV40 do not usually replicate in the cells which they transform and foci of transformed cells in culture develop as clones from the initial transformant. To test whether this is also true of human warts, as seems likely, Murray *et al.* assayed the glucose-6-phosphate dehydrogenase present in warts taken from six American negro women who were known to be heterozygous for two electrophoretically distinguishable variants (designated A and B) of this enzyme. The cells of such women fall into two classes; some have the A form of G6PD and others have the B form of the enzyme. But of the six warts taken from the six heterozygous women, four had the B form, two had the A form of the enzyme, and none comprised mixed populations of the two classes of cells. This finding surely eliminates the possibility that the growth of warts depends on the spread of wart virus from cell to cell, for if that were the case at least some of the six warts from the heterozygotes would be expected to contain cells of both phenotypes.

Murray *et al.*'s conclusion is, in fact, precisely that

expected if each wart was a clone of cells derived from a single cell transformed by human wart virus, but until a larger sample of such warts has been analysed two other possible explanations cannot be dismissed. The warts may have developed not from a single transformed cell but from a patch of transformed cells, all of which happened to have the same G6PD phenotype. Alternatively as the warts grew cells of one of the two phenotypes may have been completely eliminated by some undefined selection pressure. Both these suggestions, however, lack the simplicity and attraction of the notion that warts have a clonal origin.

Age of Fra Mauro Revised

IT now seems that NASA has been guilty of misrepresentation in putting forward the Apollo 14 landing site in the Fra Mauro formation as containing some of the oldest rocks likely to be found on the surface of the Moon. Far from revealing ages older than the 4.5 thousand million years of some of the other Apollo samples, Gerald R. Wasserburg has reported that four rocks from Fra Mauro examined so far in his laboratory at the California Institute of Technology are between 3.85 and 3.95 thousand million years old. It is true, of course, that nobody considered the low hills of Fra Mauro to be akin to the true highland regions of the Moon which are believed to represent the original, but heavily scarred, surface of the Moon. But the way in which the Fra Mauro hills extend radially away from the perimeter of Mare Imbrium—the largest of the circular maria—legitimately supports the view that Fra Mauro is composed of debris cast onto the surface when Mare Imbrium was formed. As such, rock collected at Fra Mauro could well have come from as much as 100 miles beneath the surface.

What the new ages determined by Wasserburg and his colleagues must refer to, then, is the date of the presumably catastrophic event which formed Mare Imbrium and turned the dating clock back to zero. The surprise is that it occurred so recently. Opinion has it that the mare regions of the lunar surface, such as the Mare Tranquillitatis landing site of Apollo 11 and the Oceanus Procellarum landing site of Apollo 12, must have been formed early in the history of the Moon, as likely as not all of them earlier than the age of 4.6 thousand million years determined for some of the other Apollo samples. Of course, as people will now say, the notions on which this view is based are far from watertight. Factors such as the relative distinctness with which the perimeters of the circular maria are defined come into the dating arguments, for example, and it may be relevant now that Mare Imbrium appears to be more distinct than some of the others. But if Wasserburg and his colleagues are right—and they have rightly built up a reputation in recent months for lunar age determinations that allow for many of the uncertainties that are inherent in this branch of selenology—it now seems that the processes that shaped

the surface of the Moon were active for longer than people have suspected. There has been a hint of this already in the spread of ages of material returned from the Apollo 11 and 12 flights—from 3.3 to 4.6 thousand million years—but few would have hazarded that the processes responsible for the formation of large scale features such as Mare Imbrium would have been much younger than 4.5 thousand million years.

What does this mean for the future management of the scientific aspects of the Apollo programme? Most notably, of course, it underlines the absence so far of any sample from highland areas. This ought to be rectified during the Apollo 15 flight which sets out for the Hadley rille later this month. The astronauts ought to be able to collect some highland material from the Apennine Mountains which border the rille. Yet the Apennine Mountains make up a segment of the perimeter of Mare Imbrium—will their association with Mare Imbrium once again lead to a surprisingly young age? If so, the search for material representative of the original surface of the Moon will be left, disconcertingly late, to the Apollo 16 mission to Descartes, a highland region near the centre of the visible face of the Moon, and to Apollo 17 the target of which has not yet been decided.

On the face of it, the new discovery by Wasserburg and his colleagues also has implication for views of the nature of the early solar system. Of course, it is by no means generally accepted that the circular maria were formed by the impact of what are called planetoids (in other words, large asteroids), but, if they were, the implication is that planetoids were still roaming the solar system long after most people assume that they had all been swept up by collisions. There are still a deal of rough edges inherent in this view of the origin of the maria, however. A solid object with a size of the order of the diameter of Mare Imbrium would have to dissipate an unbelievable amount of energy if it struck the Moon from solar orbit. The planetoid would undoubtedly be vaporized, and the spin and orbit of the Moon would be affected. Could the planetoid then have been in orbit around the Earth originally, leading to a smaller relative velocity at impact? Could a less dense object such as a comet be responsible? Or is there a different explanation for the maria?

MARINE BIOLOGY

Channel for Sea Snakes

from a Correspondent

ONE of the untoward ecological consequences of the proposed excavation of a sea level canal across the Isthmus of Panama might well be the colonization of a large part of the Atlantic Ocean by the yellow bellied sea snake *Pelamis platurus*. According to Graham, Rubinoff and Hecht (*Proc. US Nat. Acad. Sci.*, **68**, 1360; 1971), the relationship between the present distribution of this venomous marine snake in the tropical zones of the Pacific and Indian Oceans and the isotherms of surface waters clearly indicates that the species is contained within the waters delimited by the 18° C isotherm. Assuming that this isotherm marks the

limits of the potential distribution of this species, Graham and his colleagues have estimated the regions of the North and South Atlantic which *P. platurus* might colonize once it had crossed the proposed new Panama canal. They conclude that, once established, the snake might well be able to extend its range during summer months as far north as Cape Cod and the mouth of the English Channel.

In the Pacific and Indian Oceans *P. platurus*, a weak swimmer, drifts in surface waters feeding on the small fish in the drift line debris. The warm Kuroshio Current takes it as far north as the southern coast of Siberia and the East Australia Current carries it as far south as Tasmania. It is carried across the Indian Ocean by the Agulhas Current but the cold Benguela Current effectively blocks its penetration of the

Kin to the Fowl

STUDENTS of molecular evolution will find food for reflexion in two articles about the primary and tertiary structure of lysozymes in *Nature New Biology* next week. Canfield *et al.* show in the first place the sequence of a human lysozyme, occurring in massive quantity (1–2 g per day) in the urine in a form of leukaemia. The similarity to hen egg white lysozyme is obvious and the homology amounts to some 60 per cent of the total sequence. There are four disulphide bridges just as in the hen egg enzyme. The similarity extends to the tertiary structure, where indeed it is much more marked: Blake and Swan, in the companion article, have obtained a 6 Å Fourier map of the human lysozyme structure, which is adequate to allow a fairly detailed comparison.

The four α -helical segments of the chain are clearly defined and the rather extended linking regions also seem to be closely similar in the two enzymes. When the inhibitor, N-acetylglucosamine, is introduced into the crystal, the electron density difference map reveals only the single binding site at the active centre cleft, with almost exactly the same geometry as in the hen egg enzyme. The bulk of the 40 per cent of substitutions occur in parts of the chain that are in contact with the solvent, according to the now well established rule, and one amino-acid deletion, as well as an insertion, referred to the hen egg lysozyme sequence, are readily accommodated in an extended part of the chain. At the active site cleft there is substantial conservation of sequence, even though a good part of it is open to the solvent. Some changes at the active site are to be seen, however: the presumed catalytic groups are preserved, but there are four sub-

stitutions among the fifteen residues that make contact with the substrate. Most strikingly, one of the pair of adjacent tryptophan residues, that are a feature of the active site in hen egg lysozyme, is replaced by tyrosine, and this is expected to cause the loss of one of the hydrogen bonds securing the substrate. Where the substitutions seem to be most felt is in a region of rather poorly defined binding of the sugar, where the remaining three occur. The important implication of this work, however, is the close similarity of the two proteins, rather than the fine points of difference. From sequence data at present available it seems that human milk lysozyme, and duck and turkey lysozymes, will also turn out to be closely similar, and the homology with the α -lactalbumins is of course now well known.

The real surprise comes in the second part of the article of Canfield *et al.*, in which they report the sequence of the first thirty residues—nearly a quarter of the molecule—of goose egg lysozyme. Suspicion about this enzyme had been roused when it was reported not to interact with N-acetylglucosamine, and indeed the rules of evolutionary kinship seem to have been violated, for the partial sequence is utterly unrelated to that of hen (or duck and turkey) egg lysozyme. It has been previously found that swan egg white has two distinct lysozyme species, which cross-react antigenically with hen egg and goose egg lysozymes respectively. Evidently then two unrelated genes for lysozymes must exist. The goose sequence, it may be noted, is equally unlike papaya or phage lysozymes, which are something different again. Evidently there are more ways than one of cooking a cell wall.

South Atlantic beyond the Cape of Good Hope.

Although, as Graham *et al.* have shown with captive specimens, the snake can tolerate temperatures as low as 5° C for about an hour, when it is rapidly cooled to about 16–18° C it stops feeding and does not acclimatize to 17° C. The snake probably could not therefore colonize waters colder than about 18° C but it would probably flourish in the tropical zones of the Atlantic. Drifts of Sargasso weed, for example, would seem to be an ideal feeding ground.

And as the regular readers of *Nature* will know, once in the warm waters of the Atlantic there would be a strong selection against predators inclined to attack the snake. For in a fascinating study, Rubinoff and Kropach (*Nature*, **228**, 1288; 1970) concluded that naive Atlantic predators to *P. platurus* might eventually become as adverse to this species as the predators now exposed to it in the Pacific. In experiments in which they exposed predatory fish from the Pacific and Atlantic to the snake they observed that, whereas Pacific fish made no attempt to eat *P. platurus* even when they were in a feeding frenzy, Atlantic fish had no such inhibitions. But roughly one in ten attacks on the snake resulted in the death of the predator either from external or internal bites and occasionally, Jonah-like, the snake was regurgitated alive. Rubinoff and Kropach, who believe the avoidance reaction of Central American fish has probably evolved to its present pitch in less than 3 million years, argue that although Atlantic predators might slow the rate of colonization they would be unlikely to prevent it and selection for predators with an avoidance reaction would be great indeed.

PROTEIN SYNTHESIS

Catching a Polysome

from our Molecular Biology Correspondent
WHERE there are no operons messengers are evidently monocistronic. This raises the possibility of plucking out of a cell extract a polysome fraction engaged in the synthesis of a single protein. An unsubtle, but widely applicable, method is to separate the polysome population by size in a sucrose gradient. This has been used to good effect by Rich and his colleagues for isolating fractions from embryonic muscle associated with the production of different structural proteins. In their most recent article Low, Vournakis and Rich (*Biochemistry*, **10**, 1813; 1971) have succeeded in localizing a polysome fraction that seems to give rise to myosin light chains, the small proteins present in the myosin "heads", one of them,

as some workers believe, being inseparable from the ATPase activity.

The sucrose gradient sedimentation profile of the embryonic muscle extract shows one rapidly sedimenting peak, a broader peak, apparently containing several unresolved components, and a monosome band. Fractions from this profile are allowed to synthesize labelled protein, and the product is mixed with carrier myosin and chromatographed on an ion-exchange column. Only the radioactivity from the heaviest resolved polysome fraction follows the myosin zone. This remains true in polyacrylamide gel electrophoresis, where it is also seen that the counts from the highest polysome fraction superimpose on the zones corresponding to the two major light chains, both in the presence and absence of detergent—that is to say when migration depends on the charge of the protein, or on the molecular weight. When the myosin, in the presence of the labelled product from the low-molecular weight polysome fraction, was passed through a cycle of salt concentrations, known to cause dissociation and then reasso-

ciation of the light and heavy chains, the counts became incorporated in the myosin zone. A corresponding light polysome fraction from the embryonic liver generated labelled proteins which did not follow the light chain profile or hybridize with myosin.

Low *et al.* have thus knocked down the Aunt Sally which they erected at the beginning of their article that the light and heavy chains might have been synthesized on one polycistronic messenger, or that they might derive from a single precursor chain (a "promyosin"). Their aim is to establish whether in normal cells the light chains turn over more rapidly than the heavy chains, which are involved in the architecture of the thick filaments. The answer is promised for another day.

Miller, Cuatrecasas and Thompson (*Proc. US Nat. Acad. Sci.*, **68**, 1014; 1971) in devising a chemical affinity method for catching their polysome have put their trust in the rapier rather than the bludgeon. Their interest lies in the control of synthesis of the enzyme, tyrosine aminotransferase, which is made in mammalian tissue cul-

Recognizing Individual Chromosomes

In last weekend's *Nature* (**231**, 503 and 532; 1971), Rowley and Bodmer and Yunis and his colleagues described methods for identifying individual chromosomes using Giemsa stain and quinacrine mustard which reveal the characteristic distribution of bands of heterochromatin in chromosomes. In next Wednesday's *Nature New Biology*, Sumner, Evans and Buckland and Gagné and Tanguay report their variations on this fashionable theme.

Sumner and his colleagues claim that their modification of the Giemsa procedure — they fix mitotic cells in methanol and acetic acid and after air drying incubate the cells in sodium saline citrate solution for 1 h at 60° C before staining with Giemsa for 1.5 h — is cheaper, simpler but as effective as the quinacrine mustard technique for identifying individual human chromosomes, with one exception. The exception is the recognition of the human Y chromosome in interphase nuclei which is not revealed by the Giemsa procedure but can be recognized by quinacrine mustard fluorescence.

Sumner *et al.* have shown that during fixation the chromosomal DNA is denatured only to reanneal partially during air drying. Regions rich in repetitive sequences reanneal more perfectly than do regions containing non-repetitive sequences; as a result the bands of repetitive DNA resist the second round of denaturation during incubation in saline citrate and are preferentially stained by

the Giemsa, which preferentially binds to double stranded DNA.

Sumner and his colleagues note, however, that the number and distribution of bands of heterochromatin stained by Giemsa do not precisely correspond to the known amount and distribution of human satellite DNA, which suggests that the binding of Giemsa stain may depend on the base composition as well as the secondary structure of DNA. Gagné and Tanguay, on the other hand, using a slightly different staining regimen, state that the Giemsa stain probably stains all the repetitive DNA irrespective of base sequence whereas the quinacrine mustard is more specific with binding more dependent on sequence. By using the two stains sequentially on the same chromosome preparations they have identified two sets of heterochromatin bands; one is stained by both Giemsa and quinacrine mustard, the other only by Giemsa. This finding is, of course, compatible with reports that there are at least two sorts of human satellite DNA which differ in base composition.

But in spite of these minor discrepancies between the observations of the various groups who have used these stains, discrepancies which may well stem from small differences in the staining procedures, cytogeneticists now have the tools which they have hankered after for so long. Now they can reliably identify individual chromosomes a rich harvest of discoveries can be anticipated.

ture cells, at a rate that can be modulated by steroids. This enzyme requires as a cofactor pyridoxal or pyridoxamine phosphate, and although it seems to be generally the case that an enzyme becomes functional only when its chain is essentially complete, the structural requirements for cofactor binding are evidently altogether less stringent. That the nascent chain can be trapped on an agarose column by specific interaction with covalently attached pyridoxamine phosphate groups is an extraordinary slice of luck.

At all events the required polysomes are selectively stripped out of the cell supernatant on such a column. Only 1 per cent of all polysomes were selectively trapped, and these showed greatly enhanced tyrosine aminotransferase synthesis, as detected by immunoassay, the fraction of total new protein represented by this enzyme being measured in terms of the label brought down in the immune precipitate. Enzymatic activity was also measured. It was shown that non-specific adsorption on the column led to no increase in synthesis, and that cycloheximide inhibited the appearance of the enzyme in cell-free systems prepared from the purified polysomes. The isolation of such an enriched fraction, specific for synthesis of the one enzyme, clearly gives the authors a flying start in their aim of clarifying the mechanisms of action of the steroid hormones, if these indeed exert their control at the translational level.

APHIDS

Conifer Feeders Identified

THE life cycle of plant lice or aphids is notoriously complicated because it involves several distinct stages or morphs and one or more plant hosts. In its simplest form it includes winged and wingless generations which alternate through the summer months reproducing parthenogenetically, till the autumn when males are produced; these mate with the females and produce fertilized eggs that overwinter before a sexual reproduction starts again in the spring.

Because so many aphids are serious pests, it is obviously important to be able to identify the different morphs of a particular species. This is often easier said than done. In the case of the woolly aphids, the Adelgidae, specific identification is very difficult or even impossible and in Europe only a dozen or so species of these serious pests of conifer trees have been recorded, though it is relatively easy to place these insects into one or other of two genera, *Pineus* and *Adelges*. Considerable help towards the identification of the winged forms (alatae) of adelgids is now given in a new booklet by C. I.

Carter of the British Forestry Commission (*Bull. For. Comm.*, No. 42; 1971). This publication includes morphological keys and descriptions of fourteen winged morphs of the nine adelgid species having alatae found in Britain, and, for silviculturalists outside Britain, there is also a world check list of adelgids, their common hosts and distribution. Three of the species described, *Adelges viridana*, *Pineus pineoides* and *P. orientalis*, are recorded for the first time in Britain.

Like other members of the superfamily Aphidoidea, adelgids are soft bodied, have paired membranous wings and feed on plant sap; but in many

other respects adelgids differ from true aphids (family Aphidae), in that they have short antennal segments, a reduced wing venation, no siphunculi, and all the female forms are oviparous. The life cycle of adelgids is decidedly more complex than the basic pattern described earlier, and there is much variation between species. The cycle usually takes two years to complete and involves five distinct morphs. All but one of the female forms are parthenogenetic. In most adelgids, the winged forms migrate from the primary host, always a spruce (*Picea*) to the secondary host, another conifer which may be a silver fir (*Abies*), a larch

Single Domain Limits of Haematite

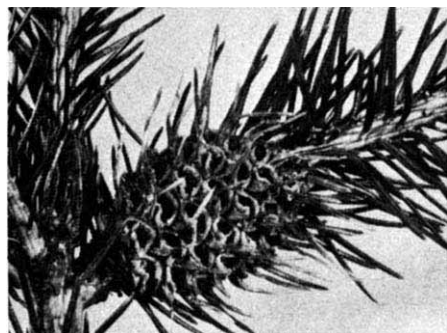
IN spite of its many achievements palaeomagnetism is still largely a phenomenological discipline—that is to say, it is possible to derive from palaeomagnetic data many conclusions which are for the most part consistent and geologically sensible without enquiring too closely into the detailed physics of the rock magnetizations. Indeed, it is perhaps fortunate that this is so, for if scientists had insisted on clearly understood magnetic mechanisms as a precondition for the acceptance of palaeomagnetic data it is doubtful whether the past decade's revolution in the Earth sciences could have taken place at all. Of course, none of this is to suggest that the basic solid state physics of rocks is unimportant, nor that to learn more about how rocks become magnetized is undesirable or unnecessary. The fact of the matter is, however, that rock magnetism—as opposed to palaeomagnetism—is an extremely difficult subject to tackle, not least because rock magnetizations are usually contained in small, impure and inaccessible grains and not in the laboratory pure, easily manipulated forms more familiar to the “pure” solid state physicist.

Perhaps the best example of the gap between complete understanding and extreme usefulness is haematite. Haematite is self-evidently an important carrier of magnetization, especially in sediments but also in certain types of igneous rocks; and yet to this day the reason why haematite is magnetic at all is still not firmly resolved. Two things about haematite seem to be quite clear, however. First, there is a certain grain size below which haematite becomes superparamagnetic and thus highly unstable magnetically. In this condition it is of little palaeomagnetic use. The second fact is that above the superparamagnetic region there is a large range of grain sizes within which haematite exists as single domain grains. Thus whereas haematite magnetizations tend to be weak compared with, for example, the

equally important titanomagnetites, the single domain condition makes for extremely high stability—a property which is, of course, extremely useful bearing in mind that many rocks were first magnetized thousands of millions of years ago. Above the single domain range there is a point at which haematite forms multi-domain grains which are again magnetically less stable; but in so far as very little haematite probably exists in this form this transition is less important.

To determine just what the two grain size translocation points are is an extremely intractable problem; but in next Monday's *Nature Physical Science* Banerjee attempts to bring some firm experimental evidence to bear on a field which is rife with conflicting data. Data published some years ago by Creer (*J. Geomagn. Geoelect.*, **13**, 86; 1962) suggest that the superparamagnetism to single domain transition occurs at grain sizes less than 100 Å. But Strangway *et al.* (*Geophys. J.*, **15**, 345; 1968) placed the same transition at 5000 Å, Kundig *et al.* (*Phys. Rev.*, **142**, 327; 1966) put it at 150 Å, and Stacey (*Adv. Phys.*, **12**, 45; 1963) made a theoretical estimate of 1800 Å. Banerjee's new magnetic experiments on synthetic haematites, which were designed to avoid some of the less desirable aspects of previous experimental work, give a clear transition at 275 ± 50 Å; and this value is unlikely to be disputed for a long time to come.

Banerjee's determination of the single to multi-domain transition is based not on new experimental evidence at all but on newly interpreted data from an old article by Chevallier and Mathieu (*Ann. Phys.*, **18**, 258; 1943). A plot of coercive force against grain size clearly shows the power law behaviour typical of multi-domain grains above 150,000 Å but not below. This grain size, Banerjee suggests, should thus be taken as the best estimate of the transition from the single to the multi-domain condition.



Open gall of *Adelges abietis* formed on a shoot of *Picea sitchensis*.

(*Larix*), a pine (*Pinus*), a Douglas fir (*Pseudotsuga*) or a hemlock (*Tsuga*).

The wingless or apterous forms occur only on the primary host *Picea* in the summer. Each fertilized female of this so called sexuales generation lays one egg which later hatches to give the fundatrix—the first wingless female of the cycle. The fundatrix nymph settles down on the current year's shoot, usually near to a bud where it overwinters. By April of the following year, the fundatrix is mature and it lays a large number of eggs which hatch to give the so called gallicola nymphs. This is the stage associated with the formation of the characteristic conifer galls or false cones (see illustration); the nymphs crawl into the buds and while they feed on the sap and the young tissue galls form around them.

CANCER THERAPY

European Unity

from a Correspondent

At the beginning of the fifth plenary session in Paris on June 25 of the European Organization for Research and Treatment of Cancer (EORTC), the enlargement of the European Economic Community was anticipated and, with it, the emergence of a continental sized responsibility in science and medicine. Professor H. J. Tagnon (Institut Jules Bordet, Brussels) urged the establishment in Europe of a medical research organization comparable in scale and influence to the National Institutes of Health in the United States. His plea that European governments should negotiate urgently to fund such a development was followed up by Professor D. W. van Bekkum (Radiobiological Institute, Rijswijk, Holland) who read an open letter sent to west European governments by the council of the EORTC.

During the first scientific session, carcinoma of the breast was exposed from a variety of angles. Mr J. L. Hayward (Guy's Hospital, London) presented evidence from a large series which clarified the remaining issues in the long-standing controversy of con-

servative versus radical surgery. Radical mastectomy seems to confer only greater freedom from local recurrence and this is at a price in physical and cosmetic complications which may be too high. Although a trend toward treatment which is tailored specifically for the individual seems to be emerging as the natural sequel to the studies reported by Professor N. Veronesi (National Cancer Institute, Milan), the number of variables and the time scale of the disease are such that the answers will come slowly with even large scale cooperative trials.

Controlled clinical trials were criticized and stoutly defended in the following session. There may be good reasons why certain centres should not participate in such trials but the generality of opinion and evidence was firmly in their favour. Some of the progress in cancer therapy reported

had been achieved by repeated marginal advances which could only be defined and confirmed by properly designed trials, and if they cut down to size some of the more extravagant claims of uncontrolled trials this hardly seemed a reason to abandon them.

Dr G. W. Barendsen (Radiobiological Institute, Rijswijk, Holland) demonstrated the wide range of techniques necessary to elucidate cell kinetic data for a rat rhabdomyosarcoma and the way such data could be used to secure more effective treatment. Little optimism could be aroused for the prospect of obtaining comparable data for human tumours and there was no certainty that it could be utilized if it were. The best hope seemed to be that an index of viable cell mass, comparable to that already available for human choriocarcinoma, could become more commonplace as

Radio Emission from Extensive Air Showers

THE study of cosmic rays of very high energy, 10^{15} eV and above, is practicable only because on entering the Earth's atmosphere they produce cascades of secondary particles—the extensive air showers. The lateral spread of the secondary particles over many hundreds of metres makes possible the detection of showers on a collecting area of tens or hundreds of square kilometres with an array of particle detectors laid out in a suitable pattern on the ground. The total area of the particle detectors may be only a few hundred square metres.

Large particle detecting arrays are at present the only way to study the very rare showers initiated by primary particles of energy greater than 10^{19} eV (approximately 1 J). Data from the arrays now operating in the United Kingdom (University of Leeds) and in Australia (University of Sydney) suggest that the energy spectrum of the primary cosmic radiation above 10^{19} eV is just a smooth extrapolation of the trend at lower energies. This is surprising, because it had been expected that interactions between the cosmic ray particles and the 3 K black-body background radiation would lead to a cutoff in the spectrum at $\sim 10^{19}$ eV. New techniques for the study of large air showers are urgently being sought in the hope of clarifying this discrepancy.

One promising line of development is the study of the radio emission from an air shower. Radio pulses in time coincidence with air showers have been detected at frequencies between 2 MHz and 500 MHz. The radiation has its origin in the earlier stages of the shower development, high in the atmosphere, and it therefore carries information supplementing that given by particle detectors on the ground. Some workers

in the field believe that a comparison of the particle and radio information will make it possible to set limits on the mass of the incoming primary cosmic ray particles.

There is, however, a serious difficulty in applying the radio technique to the largest showers. At the frequencies most commonly used (20 to 100 MHz), the radiation is concentrated within 100 or 200 m of the shower axis; an extremely large number of radio receivers would therefore be needed to cover effectively the collecting area of a large particle array. Because it seems that the lateral spread of the radiation may vary inversely with the frequency, there is a strong incentive to develop low frequency techniques.

In next Monday's *Nature Physical Science*, a communication from Prescott's group at the University of Calgary describes useful progress in this direction. They note that there is an optimum observing frequency near 3.6 MHz because the D-layer of the ionosphere gives strong daytime absorption of unwanted interference. Not having access to a large particle detecting array, the group have had to content themselves with studying small air showers, of energy between 10^{15} and 10^{16} eV, for which the radio emission is still less than the background. Nevertheless, by ingenious use of computerized data analysis, they were able to extract information free from observational bias and show that the field strength at 3.6 MHz is an order of magnitude greater than it is above 20 MHz. If this high field strength is combined with a wide spread about the shower axis, the low frequency radio emission may prove to be an ideal way of studying very large air showers.

soluble products from other tumours become measurable.

At the end of the final session, Dr R. C. Gallo (National Institutes of Health, Bethesda) summarized the current state of play with reverse transcriptase. Only one virus not known to be oncogenic has been found so far to possess the enzyme and he felt it could still be relevant to neoplasia even if not specific to tumour cells. He also brought news of a type C virus isolated from an American Burkitt lymphoma which is associated with RNA dependent DNA polymerase, and as a final titbit he reported that by screening more than a hundred rifamycin derivatives, a small number with high anti-polymerase activity had been obtained.

MARS

Microwave Spectrum

from a Correspondent

THE spectrum of the microwave radiation from the planet Mars is a fascinating enigma which is still poorly understood. A controversy over the correct interpretation of the spectrum is exposed in the current issue of *Icarus* which carries several mutually exclusive research notes. The simplest prediction, based on the equation of heat conduction, is that the measured brightness temperature of the planet will show an increase at shorter wavelengths. This is because long waves record the mean planetary temperature, whereas shorter waves penetrate to shallower depths which are heated up by the solar radiation. In principle the location of the spectral turn up expected somewhere in the millimetre wave region gives information on the thermal and electrical properties of the surface, but, unfortunately, the observations will not fit this naive pattern.

In such a confused situation it is gratifying that several groups are striving to improve the quality of the data, particularly in the critical zone below 1 cm wavelength. Observations made during the 1969 opposition at the Crimean Astrophysical Observatory yield temperatures of 240 K at 2.3 mm and 210 K at 8.15 mm. These values are considerably higher than those obtained in other experiments, and in fact V. A. Efanov *et al.* (*Icarus*, 14, 198; 1971) claim them as evidence for a temperature enhancement at short wavelengths. In order to do this, however, they have arbitrarily to discard some of the data.

Contrasting with this result, a similar measurement at 8.22 mm by A. D. Kuz'min *et al.* of Moscow (*ibid.*, 192) gave a temperature of 176 K which is indicative of a turn down in the spectrum. When these authors attempted to relate other data to this point it became obvious from the many contra-

dictory measurements that no simple conclusion is possible. Falling neatly between the sets of Russian measurements is a further result by R. W. Hobbs and S. L. Knapp of the Naval Research Laboratory, Washington, who obtained 207 K at 9.55 mm wavelength (*ibid.*, 204) from which they conclude that the temperature is constant over a range from 1 mm to 21 cm.

In a critical reassessment of the published data, including the forementioned results, E. Epstein concludes (*ibid.*, 214) that the spectrum definitely does not turn up at short wavelengths as predicted by elementary theory. He has reduced to a common basis all measurements made with sensitive equipment. Furthermore, he has taken into account systematic errors caused by uncertainties in the flux densities of the primary calibrators and has rejected points with large errors. The final result of this weeding out process is a flat or slightly turned down spectrum, although the errors on individual points (typically ± 30 K) are as large as the reduction at short wavelengths.

The final article, by Carl Sagan and Joseph Veverka (*ibid.*, 222), is an objective examination of Epstein's conclusion that the simple theory must be incorrect. There are several ways of changing the argument by introducing internal sources of heat, ferromagnetic materials, differing electrical and thermal skin depths or radiative conduction, so that the heat budget of the planet is modified. None of these artificial devices will, however, reproduce Epstein's spectrum.

According to Sagan and Veverka the observed spectrum is obtained if there is a thin layer of material with high dielectric constant and high absorption coefficient close to the Martian surface. This layer must be entirely transparent to centimetre radiation, but strongly influence millimetre wave propagation. It is not very surprising to learn that a layer of liquid water on Mars would have just these properties. The total volume of liquid water needed—a layer 50 μ thick—is not much larger than the amount already present in the tenuous atmosphere as vapour.

Membrane Bound Polyribosomes

WITHIN the cytoplasm of animal cells a fraction of the polyribosomes may be topologically segregated from the rest by attachment to membranes. This is particularly evident in tissues which secrete large amounts of protein, such as liver, pancreas and plasma cells, where ribosome-studded "rough endoplasmic reticulum" is most abundant. The immediate inference is that the ribosomes attached to the endoplasmic reticulum are largely those synthesizing proteins destined for streamlined package and export, and during the past few years data have been accumulating in support of this idea. For example, in liver, membrane bound polysomes synthesize predominantly serum proteins and free polysomes synthesize non-serum proteins such as ferritin (Ganoza and Williams, *Proc. US Nat. Acad. Sci.*, 63, 1370; 1969; Hicks *et al.*, *Science*, 164, 584; 1969). An apparent prerequisite for the correct assembly of the light and heavy chains of immunoglobulin in lymph node cells is their discharge from membrane bound polysomes into the endoplasmic reticulum (Vassalli *et al.*, *J. Mol. Biol.*, 56, 1; 1971).

Although this evidence confirms pre-conceived ideas, it does raise some important questions about the mechanism of assembly and selective attachment of these polysomes to the endoplasmic reticulum. A report by Baglioni, Bleiberg and Zauderer in next Wednesday's *Nature New Biology* is the vanguard of what promises to be an all-out attack on these problems. Working with mouse

myeloma cells which secrete a single immunoglobulin and have a well developed endoplasmic reticulum, Baglioni *et al.* consider three models for the assembly of membrane bound polysomes. These models incorporate the fact that polysomes are attached to the endoplasmic reticulum through their 60S subunits, from which the nascent polypeptide chains probably extend towards the lumen, and the generally accepted idea that ribosomes join messenger RNA in the sequence 40S ribosomal subunit plus "initiation factors", followed by the 60S subunit.

In the first model ribosomes translating mRNA coding for an exported protein become attached to membranes only when the nascent polypeptide chain is long enough to recognize specific binding sites. Conversely, the mRNA-80S initiation complex may bind to the membrane immediately because of some specificity in the 60S subunit, or an mRNA-40S complex may seek out a 60S subunit already membrane bound.

Baglioni *et al.* distinguish between these alternatives by using a double isotope labelling technique to observe the entry of newly synthesized ribosomal subunits into the membrane bound and free ribosome populations. By carefully eliminating contamination of the first fraction by mitochondrial ribosomes and subunits they show that new 60S subunits become attached to membrane independently of 40S subunits and in the absence of protein synthesis, thus eliminating models one and two.

LIBRARIES

An Accident of History

from a Correspondent

LIBRARIES, like collections of all kinds, are only as good as the catalogue which facilitates access to them. Without an available catalogue potential users have no means of determining whether they hold useful material. This is particularly true of those libraries on the periphery of the national reference library circuit, such as that at the Natural History Museum in London, which has published a complete catalogue of its holdings of published works up to 1939. This catalogue is now supplemented by a list of manuscripts and original drawings in the museum with a short history of the libraries compiled by F. C. Sawyer (*Bull. Brit. Mus. Nat. Hist. (Hist. Ser.)*, 4, 77; 1971).

The library of the British Museum (Natural History), *alias* the Natural History Museum, seems to have been one of the most monumental "accidents" of history, for when the natural history departments of the British Museum moved to South Kensington no provision had been made by architects, trustees or staff for a central library. Departmental libraries had been envisaged for each discipline but in the *melée* for space in the new building even these disappeared. This accounts for the discomfort still available to users of those libraries which continue to be housed in narrow, draughty and, in wet weather, leaking corridors, although the central library attained a sumptuous reading room only eighty years after the building of the museum.

This museum originally formed part of the British Museum at Bloomsbury but in 1880 the natural history collections and staff were transferred to South Kensington. Having failed to equip the new museum with a library, the trustees then logically refused to transfer automatically the scientific literature which related to the collections. This ban included the natural history books of Sir Hans Sloane, the owner of the foundation collection of the two museums, and the priceless library of Sir Joseph Banks. The net result of these acts was that the libraries of the Natural History Museum had to start almost from nothing in 1880. Their present eminence as one of the greatest libraries in the life and Earth sciences owes as much to the expertise of its librarians and the scientific staff of the museum as to their devotion to the improvement of the library.

Although the library had to be built from small beginnings it was possible in the 1880s to do so. Thus Sawyer records the purchase of Audubon's *Birds of America* for £285 in 1885 (a copy fetched £90,000 in 1969), and in

the same year Gould's *Birds of Australia* was purchased for £200 (the present value is perhaps in excess of £6,000). Not only were such books still available in some numbers and at attainable prices, but still active were many singularly generous patrons. Thus the magnificent library, chiefly of ornithology, belonging to the Marquis of Tweeddale was presented to the museum after his death; the Walsingham collection of entomological books was presented in 1901; and in 1937 the 30,000 volume library of the second Baron Rothschild (with his vast collections of specimens at Tring, Hertfordshire) was given to the nation.

These were the great patrons of the museum's libraries, but the list of manuscripts and drawings in the library records many other donations or acquisitions of interest. The manuscript collection to some extent records the history of natural history exploration; in another sense it mirrors the rise of the British Empire. Paintings made during all three of James Cook's (1728–1779) voyages, the ship's log of HMS

Challenger and Sir John Murray's diary of the voyage of 1873–1876, and, intermediate in time, Sir Joseph D. Hooker's notes on the Antarctic explorations on HMS Erebus from 1839–1843, are all preserved in the library. There are collections of illustrations or manuscripts made by the Hon. East India Company's staff in Canton, such as John Blake's plant paintings in 1766 and the Reeves's plant and animal drawings, or by sailors such as Midshipman George Raper's drawings of New South Wales in 1787. Domestic items have also been listed, such as Sir Robert Owen's papers presented by his executors (Owen was the first director of the new museum) and the correspondence of a nineteenth century keeper of zoology, Dr Albert Günther, which was purchased recently from a descendant. Documents relating to Sir Edwin Ray Lankester's stormy career as director of the museum (a post from which he was compulsorily retired in 1907) are listed beside the autograph letters of J. L. G. Krefft, also dismissed from his curatorial post in the Australian Museum.

Lattice Theory and Complex Ions

THE theory of electrostatics allows a perfectly spherically symmetrical monatomic ion to be treated as having its overall electric charge residing at the position of the atomic nucleus. This principle has been widely used in the calculation of the Madelung constants and Coulombic terms which contribute to the lattice energies of simple salts and, in this context, the ionic constituents are considered as a regular array of point charges. It is probably not correct to calculate the contribution of the more remote ions to the Madelung energy in this way using a dielectric constant of unity but, by using an empirical repulsion potential related to experimental compressibility data and dipole and quadrupole terms, the Madelung-Born-Mayer treatment has led to results for lattice energies of simple "ionic salts" which agree satisfactorily with those computed from experimental thermochemical data. The method has proved quite successful even in the case of fluorite CaF_2 , for which Weiss, Witte and Wölfel have obtained a minimum electron density along the line Ca-F of electrons $\text{\AA}^{-3}$ by a refined X-ray technique.

For ionic crystal lattices containing complex ions that do not possess spherical symmetry (even in the idealized state), the simplified electrostatic theorem must be modified and the point charge treatment altered to consider formal charges residing at the atomic nuclei within the complex ion. Moreover if the complex involves covalent

bonding, the point charges cannot be taken to correspond to the oxidation numbers of the constituent atoms. In next week's *Nature Physical Science*, H. D. B. Jenkins and T. C. Waddington report a treatment of just this kind of situation. They have shown that by using the Bertaut expansion (*J. Phys. Radium*, 13, 499; 1952), general equations for the Madelung parameter and associated energy can be derived rapidly by computer for a crystalline salt of known structure containing a polyatomic ion. The expressions for the Coulombic energy show a quadratic dependence on the charge Q placed at the nucleus of one of the atoms forming the complex ion. Using these Madelung relationships for the pleomorphic forms of calcium carbonate and making certain assumptions concerning the magnitude of repulsion and dispersion energies, Jenkins and Waddington derive the appropriate formal charge to be placed on the oxygen nuclei in the ion CO_3^{2-} , namely 0.26 electron units. The requisite formal charge distribution on other complex ions can often be obtained by calculating lattice energies of different salts containing the ion as a function of Q and relating the results through a thermochemical cycle to the enthalpy change for a given reaction. This approach also makes possible estimates of enthalpy changes for processes involving the complex ion, which are difficult to determine by direct experiment.

Prostaglandins and Aspirin

H. O. J. COLLIER

Research Division, Miles Laboratories Limited, Stoke Poges, Buckinghamshire

This article traces the background and implications of the recent finding that aspirin and indomethacin block the production of prostaglandins E_2 and $F_{2\alpha}$.

THE prostaglandins are endogenous substances that are often reviewed¹⁻⁶ and as often the subject of symposia⁷⁻¹¹. In 1969, Pickles estimated¹ that more than two hundred articles a year were appearing on prostaglandins; by now the output must be larger. Few of these articles, however, deal with the interaction of drugs with the synthesis, release or action of prostaglandins although this was the subject of a group of three articles by Vane and his colleagues¹²⁻¹⁴ in a recent issue of *Nature New Biology*. I shall discuss here the background and implications of this work.

Vane and his colleagues have shown that aspirin and indomethacin potently block the production of prostaglandins E_2 and $F_{2\alpha}$ and they propose that this effect explains some of the ways in which these and other anti-inflammatory (or antiphlogistic) acids work. Aspirin and like-acting drugs have been characterized as "anti-defensive"¹⁵ and so the proposition that they act by blocking prostaglandin production has its converse in the postulate that a function of prostaglandins is to mediate defensive reactions to noxious influences. Thus, through this interpretation of how the anti-inflammatory acids work, the defensive function that had already been suggested for certain aspects of prostaglandin action, such as bronchoconstriction^{16,17}, smooth muscle contraction¹⁸ and skin inflammation¹⁹⁻²¹, is applied more generally.

The Function of Prostaglandins ?

Whereas a hormone is a chemical messenger released from one organ—a ductless gland—and distributed in the blood to distant sites of action, a "local hormone" (or "humoral mediator") is released in active form in many places and works near the spots where it is released because it would be inconvenient and even dangerous if widely spread through the body. Because of this danger, also, the body needs ways of inactivating a local hormone, preferably near its site of action.

An active substance liberated in effective amounts near a tissue that responds to it and within reach of a means of fast inactivation is thus likely to be a humoral mediator. Several of the prostaglandins fulfil these criteria and so they might act as mediators in many parts of the body, including the male and female reproductive systems, the digestive tract, the airways, the spleen and blood vessels, the skin, the eye and the central nervous system.

A classical method to determine the function of a circulating hormone is to remove from the body the ductless gland that puts it into the blood stream. This method is impossible with a putative local hormone and the best way to decide its function is to use a drug that selectively suppresses its effects, either by breaking a link in the chain of its production, transport, release or action, or by promoting its inactivation. Such a suppressant is likely to act somewhere in this chain as a selective antagonist (pharmacological antagonism being

regarded as any interaction whereby one substance hinders the biological action or manipulation of another). For an antagonist to be useful in determining the function of a putative mediator, it needs not only to be selective but also to be active *in vivo* against the mediator chain at most of its natural sites of action.

If a suitable antagonist is found, it may help in more ways than by disentangling the function of a humoral mediator. It may also be used to treat disease, to identify an active substance of unknown chemical structure with one that has already been chemically defined, or to help elucidate the mode of action of known drugs. Selective antagonists of prostaglandin production now seem to have been available for many years in the shape of the anti-inflammatory acids.

Prostaglandins and Antiphlogistic Acids

The exploration of antagonism between prostaglandins and the anti-inflammatory acids goes back to 1964, when aspirin was tested for blockade of bronchoconstriction induced by injecting prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) intravenously in the guinea-pig, so as to distinguish $PGF_{2\alpha}$ from slow-reacting substance in anaphylaxis (SRS-A) (ref. 22). Aspirin was ineffective against $PGF_{2\alpha}$ and effective against SRS-A. Some years later this observation was extended to meclofenamate²³.

Continued exploration of the interaction between anti-inflammatory acids and substances suspected of mediating allergic reactions led in 1968 to the unexpected finding that fenamates and phenylbutazone potently and aspirin less potently blocked the contraction of human isolated bronchial muscle induced by $PGF_{2\alpha}$, while lessening neither the contraction due to acetylcholine nor the relaxation induced by prostaglandins E_1 or E_2 (ref. 24). The dose of $PGF_{2\alpha}$ needed to elicit a given response was about twelve times as great after exposure to 0.1 $\mu\text{g ml}^{-1}$ of meclofenamate as it was before. Meclofenamate was about a hundred times as effective against $PGF_{2\alpha}$ as against SRS-A, confirming the distinction between the two endogenous substances. This antagonism occurred at drug concentrations within those attained in human plasma during treatment and the relative potencies of the four anti-inflammatory drugs tested were roughly the same as they show clinically. The antagonism was not consistent, however, with the failure of aspirin or meclofenamate to block bronchoconstriction attributable to intravenous injection of $PGF_{2\alpha}$ in the guinea-pig. This inconsistency has not been resolved, but it might be caused by $PGF_{2\alpha}$, in one situation or the other, being converted into or releasing a more active bronchoconstrictor substance.

In several situations, aspirin or sodium salicylate has been shown also to antagonize arachidonic acid, which is a precursor of prostaglandins. This antagonism occurs in isolated smooth muscle²⁵, in the bronchoconstrictor response of guinea-pig lungs *in vivo*²⁶ and in the abdominal constriction response to injection of arachidonic acid in the mouse²⁷.

Another antagonism of prostaglandins by an antipyretic drug (paracetamol), albeit with little anti-inflammatory activity, has recently been described; intraperitoneal injection of paracetamol prevented or delayed fever induced by injecting $PGF_{1\alpha}$ or PGA_1 , or to some extent $PGF_{2\alpha}$, into a cerebral ventricle of the conscious cat^{28,29}. Paracetamol was unselective

enough to block also fever caused by intracerebral 5-hydroxytryptamine or TAB vaccine³⁰; but it was ineffective against the more pyretic actions of PGE₁ and PGE₂ (refs. 28 and 29).

The articles by Vane and his colleagues describe a blockade by aspirin and indomethacin, but not by hydrocortisone or morphine-like drugs, of prostaglandin production that is potent, affects both PGE₂ and PGF_{2α} and is not capricious in its incidence in three different types of preparation *in vitro* from three different species. Vane reports¹² that indomethacin, aspirin and sodium salicylate (in descending order of potency) blocked the synthesis of prostaglandins E₂ and F_{2α} from arachidonic acid in cell-free homogenates of guinea-pig lungs. Ferreira, Moncada and Vane report¹³ that infusion of indomethacin or of aspirin inhibited the production of prostaglandins E₂ and F_{2α} from dog isolated spleen, induced by adrenaline. Thus the authors show in an isolated organ the same effect as Vane finds in a cell-free homogenate. Smith and Willis report¹⁴ the complementary finding that indomethacin, aspirin and sodium salicylate (in descending order of potency), added to a suspension of human platelets, blocked the production of prostaglandins induced by thrombin, without impairing the release of endogenous substances. The platelets of blood withdrawn from volunteers who had ingested aspirin or indomethacin one hour before were also unable to produce prostaglandins in response to thrombin.

The finding that the anti-inflammatory acids can effectively break links in the chain of synthesis and action of prostaglandins, chiefly by blockade of their production¹²⁻¹⁴, but also by antagonism of PGF_{2α} (ref. 24), leads in two directions. First, it helps biologists to explore and comprehend the function of prostaglandins; second, it helps to clarify the mode of action of the anti-inflammatory acids, which will now be considered.

Blockade of Prostaglandin Production

Most drugs probably work by interfering with one or more of the endogenous macromolecules that operate living processes, including enzymes, carriers, genes, receptors responding to autacoids, storage sites and the factors that activate or inhibit them. If a drug interferes with a macromolecule that handles the synthesis or release of, or the response to, an autacoid, or other important metabolite, the drug is thought to antagonize that process or substance. Analysis of how a drug works usually proceeds in steps leading not only to the identification of the macromolecule(s) involved and the operation(s) affected, but also to explaining how the drug exerts its effect(s)¹⁵.

In a detailed analysis of the mode of action of aspirin¹⁵, based on the literature available in early 1968, I reached the general conclusion that aspirin probably acts by interfering locally in the tissues with some stage in the humoral mediation of the body's defensive reactions, one of several possible types of mediator considered being the prostaglandins. At that time, however, the ability of prostaglandins to evoke fever or nociception was unknown, although their participation in the defensive reactions of bronchoconstriction¹⁶ and inflammation¹⁹ had been proposed. In 1969, the discovery of rabbit aorta-contracting substance (RCS) and the inhibition by anti-inflammatory acids of its release from guinea-pig lungs³¹ helped to explain the antagonism by aspirin of bronchoconstriction in the guinea-pig, induced by bradykinin or SRS-A, and possibly other of its actions^{31,32}.

Injected prostaglandins are now known to have potent inflammatory^{19,20} and pyretic^{28,29} effects and prostaglandins have been detected in inflamed tissues²¹. Because of this, because the blockade was characteristic, as far as was tested, of the anti-inflammatory acids and because it occurs at drug concentrations well within those attained in plasma clinically, Vane¹², Ferreira, Moncada and Vane¹³ and, independently, Smith and Willis¹⁴ now propose that the inhibition of prosta-

glandin synthesis that they have observed explains the anti-inflammatory, antipyretic and possibly some other *in vivo* effects of aspirin and like-acting drugs.

This proposal will ultimately have to be related to the RCS mechanism, accounting for a rather isolated effect in guinea-pig lungs. As Vane implies¹², however, the two explanations will probably prove compatible when more is known. Meanwhile, some of the other questions that the attractive and largely convincing present hypothesis raises should be considered.

Although the intradermal injection of prostaglandins does not cause pain²⁰, there are three reasons for including analgesia among the actions of aspirin probably explicable by blockade of prostaglandin synthesis. First, prostaglandin E₁, E₂ or F_{2α} irritates the mucous membranes of the eye³³ or airways^{34,35} and, when injected into the peritoneal cavity of the mouse, each evokes abdominal constriction (writhing) responses, believed to show nociception (my and C. Schneider's unpublished work). Conforming with this, aspirin does not lessen pain in skin^{15,36}, elicited by noxious substances, but it does lessen pain in other sites, when elicited by similar noxae³⁷⁻³⁹. The fit between the sites at which these prostaglandins induce nociception and those at which aspirin exerts its analgesic action is therefore good. If prostaglandins do elicit pain from the skin, the argument for including analgesia among the actions of aspirin explained by blockade of prostaglandin synthesis would be weakened rather than strengthened, because aspirin is ineffective against experimental skin pain. The second reason is that prostaglandin synthesis is stimulated by conditions associated with pain, such as trauma, ischaemia and hypertonicity⁴⁰. The third reason is the good fit between the relative potencies of indomethacin, aspirin and sodium salicylate in (1) blocking prostaglandin synthesis in lung homogenates¹², and (2) suppressing the abdominal constrictions of the mouse induced by intraperitoneal injection of a noxious substance³⁸, which in these experiments was acetylcholine, a releaser of prostaglandins in some sites (personal communication from P. W. Ramwell). The potency ratios on a weight basis of indomethacin to aspirin to sodium salicylate were: for inhibition of prostaglandin synthesis—368 to 16 to 1; for antinociception by the oral route—589 to 6 to 1.

There is a difficulty in accommodating the new hypothesis, not to the known actions of anti-inflammatory acids, but rather to the actions that they are not known to have, but that may nonetheless be mediated by prostaglandins. For example, anti-inflammatory drugs are not used against threatened abortion; nor are they often given in asthma, except that amidopyrine and phenazone are common ingredients of German asthma powders⁴¹.

A possible escape from this difficulty (although it leads to another) is offered if two prostaglandins have opposed actions on the same organ. For example, prostaglandin E₂ relaxes and F_{2α} contracts bronchial muscle and so a blockade of prostaglandin synthesis would deprive the airways of both natural bronchodilator and bronchoconstrictor. Where the two prostaglandins were out of balance, the anti-inflammatory drug might have asthma-inducing or anti-asthmatic effects, both of which are recorded in the literature^{15,16}. When this route of escape is available, it is improved by the possibility that the relative proportions of different prostaglandins formed may vary with local conditions⁴⁰. Such a route of escape, however, does not seem to be available in the instance of abortion, which is induced both by PGE₂ and PGF_{2α}.

In another direction, the hypothesis that anti-inflammatory acids work by blocking prostaglandin synthesis may broaden the logical basis for their clinical use¹². For example, these drugs might be expected to relieve some types of nausea or diarrhoea, because these are common effects of infusing PGE₂ in women to induce abortion⁴².

Another difficulty arises where prostaglandins and anti-inflammatory drugs have apparently similar, rather than

opposed, effects; in the rat, for instance, premature labour is induced by sodium salicylate⁴³. Again both PGE₁ (refs. 5 and 44) and sodium salicylate⁴⁵ inhibit the mobilization of free fatty acids from fat depots in the rat. A third example of this difficulty is that PGE₁ in various preparations inhibits the effects of sympathetic nerve stimulation⁴⁶, yet noradrenaline has anti-inflammatory activity¹⁵. Hedqvist offers⁴⁶ a way out of such difficulties by finding that larger doses of PGE₁ may have effects opposite to those of smaller doses. A comparable reversal has been noted for aspirin¹⁵; for example, large doses cause fever.

An unresolved question concerns how the antagonism²⁴ by anti-inflammatory drugs of PGF_{2α} in human isolated bronchial muscle relates to the inhibition of prostaglandin synthesis and the mode of action of these drugs. At the time of reporting it, Sweatman and I wrote "This potent and selective antagonism of a prostaglandin by non-steroidal anti-inflammatory drugs also raises the question whether any of the known effects of therapeutic doses of these drugs in man, such as the lessening of fever, of pain, of inflammation and of platelet clumping in shed blood, depends on antagonism of PGF_{2α} or of an allied endogenous prostaglandin". At first sight this possibility seems to be vitiated by the newly found blockade of prostaglandin production¹²⁻¹⁴, because such a blockade would leave little PGF_{2α} to be antagonized. No blockade is perfect, however, and so the antagonism of PGF_{2α}, if it occurs in the human body as it occurs in isolated bronchial muscle, whether PGF_{2α} acts directly or by releasing a more active substance, would reinforce the effect of the drugs against PGF_{2α}, rather than against PGE₁ or PGE₂. Such a reinforcement might provide yet another exit from some of the difficulties outlined above.

Physiological Role of Prostaglandins

By a process of fitting and interlocking familiar to puzzle solvers, the hypothesis that anti-defensive drugs act by blocking the synthesis of prostaglandins tends to strengthen and broaden the converse view¹⁶⁻²¹ that mediating defensive reactions is a function of prostaglandins, although the individual shares of different members of the group are not yet clear. The bodily and cellular mechanisms that may be considered defensive are many and varied^{15,18}. They especially involve the surfaces of the body, including the mucous membranes and their underlying muscles and blood vessels, and they have not only local but also central components. For example, the contraction of the smooth muscles of tubular organs, such as the gastrointestinal tract and airways, has been seen as defensive, both in expelling or excluding noxious materials¹⁵ and in protecting the muscle itself against excessive stretch¹⁸. If, therefore, prostaglandins share in the mediation of such reactions, their many effects and wide distribution become more understandable.

Defensive reactions have analogies, too, in other processes and functions, in which prostaglandins might also play a natural part. For example, implantation of the ovum has been likened to inflammation, which might conceivably explain the presence of prostaglandins in semen. Again, abortion in some ways resembles the protective expulsion of noxious foreign materials and so the natural participation of prostaglandins in this process could be regarded as a logical extension of a defensive role.

Defensive reactions are mediated also by substances other than prostaglandins, including amines and kinins, and these are unlikely to be affected by the same drugs as affect prostaglandins, except insofar as they induce the production or release of prostaglandins or allied substances. Mediators concerned in nervous transmission will also be involved where defence has a central component. The complexity of the humoral mediation of defence explains the complexity of its interaction with drugs. If anti-inflammatory acids act by blocking prostaglandin synthesis, the extent to which a

reaction can be treated with these drugs will help to define the share of prostaglandins in its mediation, and the extent to which other mediators participate will help to determine the limits of usefulness of these drugs.

An analogy may be drawn here between prostaglandins and money. Just as the same coins may be used to effect different transactions between different individuals in different places, so one group of humoral mediators might effect different defensive reactions in different parts of the body threatened with injury or invasion. In this sense the prostaglandins would be the coinage of the body's defences.

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Plate Tectonic Evolution of Caribbean–Gulf of Mexico Region

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A geotectonic history of the American Mediterranean is presented in terms of plate tectonics. The development of this region is presented as seven time sequence reconstructions from past Palaeozoic to present times.

PREVIOUS attempts to write a geotectonic history of the American Mediterranean (Caribbean–Gulf of Mexico area) based on continental drift models^{1–3} or, more classically, the foundering of a Palaeozoic landmass⁴ need revision because of the success of plate tectonics. New data are available from recent land field work^{5–11} and marine surveys^{12–21}. Apparently, the evolution of this region is related to former plate junctions between North America, South America, and Africa and to strike-slip, extensional, and compressional motions as the new world drifted westward²². This is the theme of the synthesis presented in this article.

The geotectonic evolution of the American Mediterranean is presented as seven time sequence reconstructions which are azimuthal equidistant projections.

Reconstruction of Drift

Pre-drift reconstruction: The closure of the Atlantic Ocean at the end of the Palaeozoic according to Bullard *et al.*²³ is modified by us (Fig. 1) to show the overlaps (in dashed lines) of the Bahama platform, southern Mexico, and Central America on to Africa and South America. In our reconstruction, we have eliminated the overlaps and the oceanic areas by rotations of these cratons (Fig. 2). Three of these, Yucatan²⁴, Honduras–Nicaragua²⁵, and Oaxaca (southern Mexico)⁹, are underlain by pre-Mesozoic basement. The isthmus of Panama and the Antilles are thought to be neo-cratons (“new ground”), created in Mesozoic–Caenozoic time, and are omitted.

The Bahama platform (more exactly, the Blake–Florida–Bahama platform) is a crescent-shaped craton with a 3–5 km post-Triassic carbonate capping²⁶. Although the nature of the basement remains unknown, we assume here that it is pre-Mesozoic, thus differing from the neo-cratonic origin of Dietz *et al.*²⁷. We propose that the south-eastern Bahama spur moved eastward to its modern position along a shear now marked by

the Straits of Florida and the North-east Providence (Bahamas) Channel. This spur would then have originally filled the V-shaped gap between Africa and South America.

Our fit of the Oaxaca craton along western Mexico is speculative, but not unreasonable. In the Bullard fit, southern Mexico south of the Trans-Mexican Volcanic Belt (clearly “old ground”)⁹ overlaps onto South America. The overlapped portion (mostly the Andean foldbelt of north-western Columbia) may be partly a marginal Mesozoic accretionary belt²⁸, but the gap created would still be too small to accommodate the Oaxaca craton.

We identify the initial suture of the Gulf of Mexico by the margin of the mid-Jurassic (Collovia–Oxfordian) basin. Starting from DeSoto submarine canyon, it trends north-westward through southern Alabama and central Mississippi, westward along the Arkansas–Louisiana state line, south-westward around the East Texas Basin, and southward into Mexico^{29–31}. This model is supported by subsurface Triassic redbeds, located by drilling, which lie just inland from the basin margin³². These presumably represent taphrogenic basins associated with detachment of the Yucatan–Nicaraguan craton.

Although Fig. 2 shows some gaps, the area probably was fully closed originally. Part of the central gap may be inter-



Fig. 1 The Bullard continental drift closure of the Atlantic Ocean, modified by adding areas of cratonic overlap shown in dashed lines. The fit is made at the 500-fm isobath.

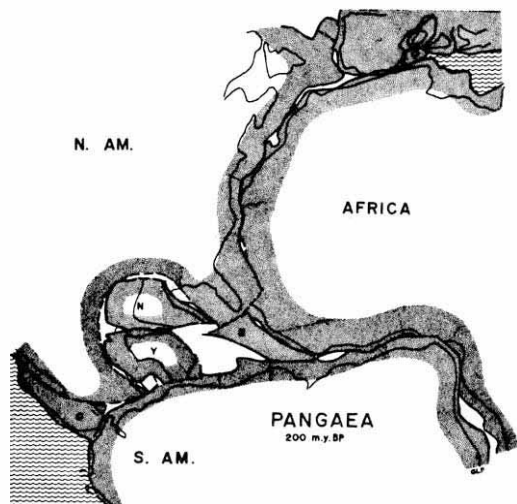


Fig. 2 Closure of the Atlantic Ocean and American Mediterranean in late Triassic (about 200 m.y. BP) according to this article. Microcontinents which are later translated are O, Oaxaca; Y, Yucatan; N, Nicaragua-Honduras; and B, south-eastern Bahamas-Blake Plateau area is accommodated by subtracting the neo-cratonic, western Senegal Basin. Continental margins are drawn to the 1,000-fm isobath, except where dashed.

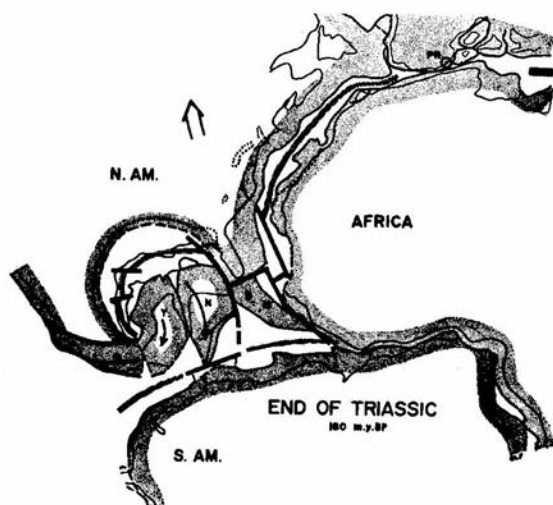


Fig. 3 Initial rifting and breakup at the end of the Triassic, 180 m.y. BP. Initial movement was rapid. Arrows are vectors showing drift relative to Africa/South America, which is arbitrarily held fixed. Dotted bands are where new oceanic crust is implaced; the dashed lines are transform faults and/or shear zones. Dotted lines on the continents outline Triassic taphrogenic basins. PR is the pole of rotation for the North American plate.

mediate crust which has been incorporated, after tectonization, into the neo-cratons of the Greater Antilles. The western part was squeezed into the Guatemala foldbelt. We would not expect a solution in the form of a simple jigsaw-fit due to the complexity of the problem.

Initial breakup: Fig. 3 shows the initial opening of the proto-North Atlantic Ocean and the pull-apart of North and South America. This is dated at or after 200 m.y. BP (late Triassic³⁴) by basalts presumably associated with this rifting³³.

The rotation pole of Dietz and Holden²² near south-eastern Spain for the opening of the North Atlantic is used with an

initial clockwise rotation of North America of 10°. South America and Africa remained joined. The split between North and South America was accomplished mostly by the opening of the Gulf of Mexico, with the Yucatan and Honduras-Nicaragua blocks rotating as a single unit about a point near the Isthmus of Tehuantepec. Sinistral shear occurred along northern South America (the El Pilar or South Caribbean shear zone). The Bahama block also started moving north-eastward.

Early Jurassic: Fig. 4 shows the plate positions at early Jurassic time (170 m.y. BP). Laurasia drifted south-westward, accommodated by sinistral shear along the Tethys seaway and the El Pilar fault zone of northern South America. Opening continued in the Gulf of Mexico. The Yucatan-Nicaraguan craton was split, creating the Gulf of Honduras sphenochasm. The Bahama craton lagged behind North America with respect to Africa and thus continued moving north-eastward. As the Gulf of Mexico, Caribbean Sea, and North Atlantic Ocean were small ocean basins at this time, lacking open circulation with the world ocean, deep-water evaporites such as the Louann were laid down.

Mid-Jurassic: Fig. 5 shows the plate positions at the end of mid-Jurassic (150 m.y. BP). As North America rotated north-westward, Newfoundland separated from Spain. Continued left-lateral shear occurred along the Tethys and El Pilar zones. The Gulf of Mexico, Caribbean, and North Atlantic remained as intracratonic ocean basins with continued salt deposition.

During the initial Atlantic opening the lack of reversals in the Earth's magnetic field is reflected in the magnetic quiet zone (MQZ). Starting at about 155 m.y. BP, however, reversals created the magnetic anomalies seen in the North Atlantic floor³⁵. The absence of anomalies in the Gulf of Mexico is mostly due to opening during the magnetic quiet time. During the last stages of opening, rapid sedimentation into the rift zone probably prevented rapid chilling of the pillow lavas which record the polarity of the magnetic field.

As the Gulf of Honduras sphenochasm completed its opening, the Yucatan and Nicaraguan cratons assumed their modern positions relative to North America. West of the sphenochasm hinge, the east-west Guatemalan foldbelt was formed, accompanied by the emplacement of serpentine bodies. Highlands produced by this orogenesis shed a thick sequence of continental and marine Jurassic sediments in Central America²⁵. We propose that this deposition extended the eastern continental margin of the Nicaragua craton and filled the western end of the Gulf of Honduras. This sedimentary wedge would later form the nucleus of the Greater Antilles.

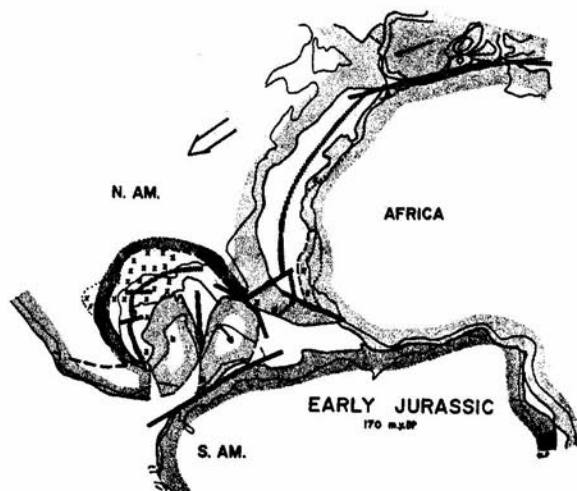


Fig. 4 Blocking out of the American Mediterranean 30 m.y. after commencement of drift. X indicates salt deposits; other symbols as in Fig. 3.

The formation of the Gulf of Mexico was complete at this stage; the Nicaraguan block had rotated about 2,500 km over 50 m.y., an opening rate of 5.0 cm/yr.

Lower Cretaceous: Fig. 6 shows the plate positions at the end of the Lower Cretaceous (100 m.y. BP). South America separated from Africa at about 135 m.y. BP and became a new plate. Both the North and South American plates encountered subduction zones (trenches) along their western margins which probably had west-dipping Benioff zones. On impingement with the continents, the zones flipped to east-dipping. Marginal orogeny with attendant volcanism ensued.

Soon after South America separated from Africa, Nicaragua separated from South America as a result of the more northerly motion of the North American plate. An open connexion to the Pacific Ocean was established. The Caribbean Sea, thus created, opened wider than it is today. Towards the end of the Lower Cretaceous, the South American plate shifted to a more northerly motion. This resulted in cessation of strike-slip motion along the El Pilar zone and the initiation of subduction, compression, and orogeny along northern Venezuela³⁶.

We propose that the Jurassic sedimentary accretionary wedge within the Gulf of Honduras (the proto-Cayman trench) and under the eastern edge of the Nicaraguan block split away from the Nicaraguan craton on both sides of the proto-Cayman trough to form the nucleus of proto-Cuba and proto-Hispaniola. As North America drifted westward, these blocks lagged behind, drifting north-eastward with respect to North America. Subduction zones formed along their northern margins causing orogeny, metamorphism of the Jurassic sediments, and volcanism. Rocks from the central gap in the original fit (Fig. 2) were also incorporated. Later, platform sediments were deposited along the northern edge of Cuba, completing the geosynclinal sequence there.

The Lesser Antilles arc was also initiated as a subduction zone, reflecting the faster rate of westward drift of the North American plate. We suggest that the Aves Ridge, located 250 km west of the modern Lesser Antilles, was the initial arc which was later abandoned by eastward migration of the Benioff zone. The plate boundary between the North and South American plates in the Atlantic Ocean is thought to be marked by several shear zones extending eastward from the Lesser Antilles¹.

Mid-Eocene: The Caribbean region attained essentially its modern aspect by the end of the Middle Eocene (45 m.y. BP)

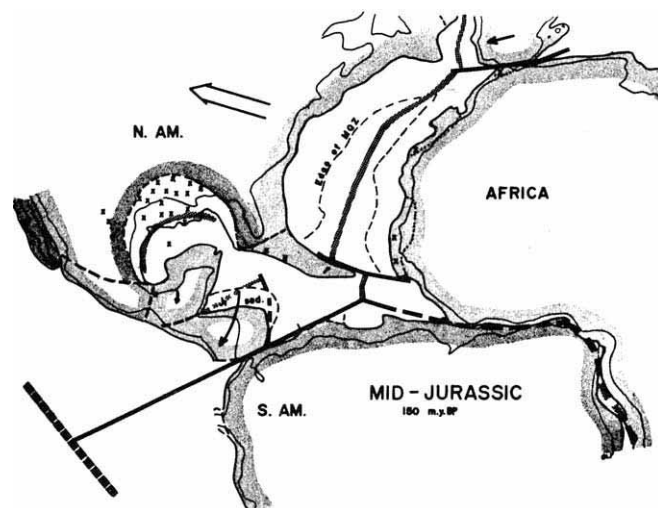


Fig. 5 The Gulf of Mexico/Caribbean near the end of the mid-Jurassic (150 m.y. BP). Note area of Jurassic sediments along the Nicaraguan craton. MQZ is the Magnetic Quiet Zone. Yucatan, Nicaragua, and the south-eastern Bahamas have reached their present position relative to North America. Heavy dashed line west of South America is a trench zone.

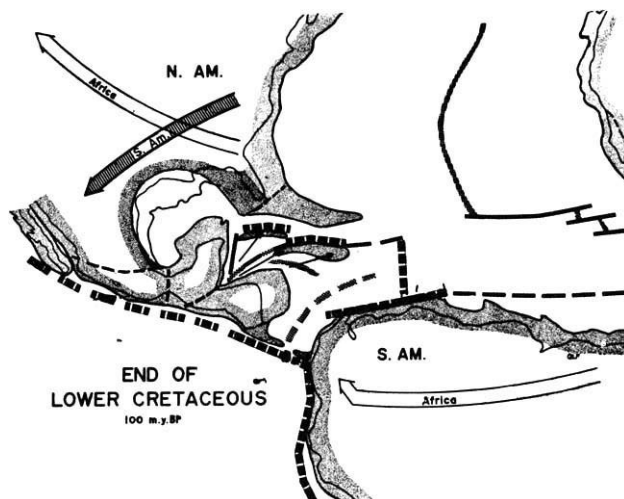


Fig. 6 Positions at the end of the Lower Cretaceous after 100 m.y. of drift. Hatched arrow on North American plate shows the relative motion between the North and South American plates; open arrows show motion relative to Africa as in previous diagrams. Heavy dashed lines are subduction (trench or compression) zones. The Jurassic sediment wedge from Fig. 5 has split off into two parts to form the nucleus of the Greater Antilles. The Lesser Antilles subduction zone is at the Aves Ridge. The Caribbean Sea is seen to be wider than it is today. Venezuelan orogenesis starts.

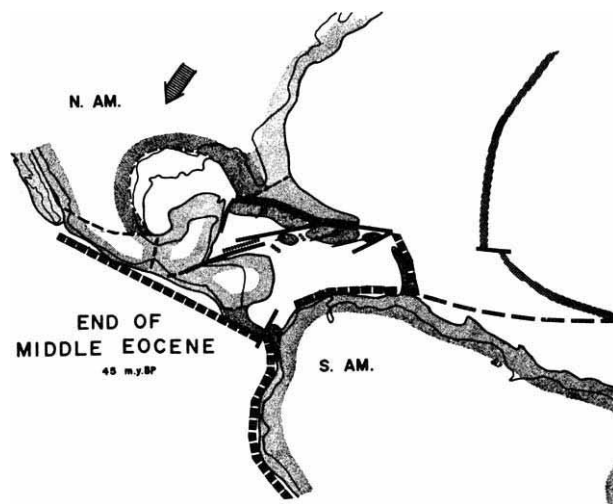


Fig. 7 The end of the mid-Eocene. The plates are essentially in their present position. The Panama twist is formed due to differential motion between the North and South American plates.

(Fig. 7). Cuba and Hispaniola completed their north-eastward relative motion. Additional spreading centres south of the Cayman trough are postulated to have split proto-Hispaniola into further numerous sub-blocks—that is, Jamaica, Hispaniola, Puerto Rico, the Virgin Islands and so on—whose edges are reflected by intermediate depths. The Beata Ridge may also be one of these. Interaction along the leading plate boundaries and within the Caribbean blocks continued orogeny throughout the Tertiary^{10,37-39}. Active subduction beneath the Lesser Antilles arc moved eastward to its modern position during the Eocene. As North America drifted faster than South America, the Caribbean region closed slightly. Continued compression along northern Venezuela and distortion of the Panamanian region resulted. Volcanism slowly closed the Isthmus land gap.

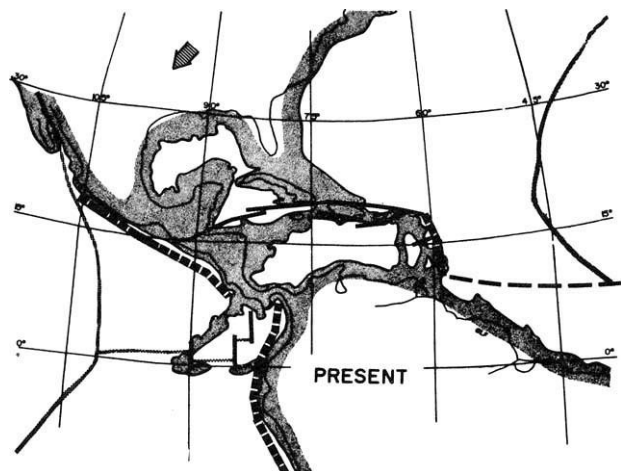


Fig. 8 Present situation. Continued motion between the North and South American plates is accommodated mainly by the Cayman-Puerto Rico shear zones, the Lesser Antilles subduction zone (which has migrated eastward), and a poorly defined shear zone extending eastward from the Lesser Antilles.

Orogeny also continued along the Pacific plate margins, responding to this subduction zone which was pushed westward along the leading continental edge.

Present: Throughout the remainder of the Cenozoic to the Present there was and is continued differential motion, interaction and closure between the two American plates (Fig. 8). We regard these two plates as distinct entities separated mainly by the Cayman-Puerto Rico megashear. Thus, the east-west plate boundary has shifted from the northern margin of South America to the Cayman-Puerto Rican shear zone. The Gulf of Mexico is part of the North American plate, while the Caribbean region is part of the South American plate. The El Pilar zone is mostly inactive today⁴⁰.

In the Neogene interaction of the North American plate with part of the East Pacific Rise caused separation and northward slippage of Baja California. The Galapagos-Panama ridge-fracture system was created within the East Pacific plate during the last 10 m.y.⁴¹.

Consequences of the Model

The foregoing is an attempt to formulate a reasonable plate tectonic history of the Gulf of Mexico/Caribbean region within the context of known geology. Some consequences of our model are as follows.

(1) The age of the sea floor crust of the region would be largely early drift—that is, early Jurassic for the Gulf of Mexico and mostly Lower Cretaceous for the Caribbean. There was a large amount of early extension and emplacement of new crust, followed by slight closure during the Tertiary. No Palaeozoic oceanic crust would be present.

(2) The Yucatan and Nicaraguan blocks are clearly old cratons which, by their movement, formed the Gulf of Mexico before the mid-Jurassic period. Movement of these blocks was completed before South America separated from Africa at about 135 m.y. BP. The Caribbean Sea attained a modern aspect by Upper Cretaceous time at 100 m.y. BP.

(3) Both the Greater and Lesser Antilles are regarded as primarily neocratonic (that is, post-breakup in origin). Metamorphism has obscured the central pre-drift “gap” material which, if present, was incorporated into the eugeosynclinal belts of the Greater Antilles.

(4) The Caribbean area is a subplate presently attached to the South American plate, with little or no movement between them at this time. It is protected from destruction by inward-dipping subduction zones on both east and west. The Gulf of

Mexico, Yucatan, Cuba, and Bahama areas are parts of the North American plate.

(5) The early differential motion between the North and South American plates was accommodated by a shear zone along the northern margin of South America. The present differential motion is accommodated by shear along the northern Caribbean margin (the Cayman-Puerto Rico trench system), and is much less than during the initial stages of movement.

(6) Unlike the solution from Bullard's fit, the region was fully closed before 200 m.y. BP so that there was no “Mare Occidentalis”—western bay indenting Pangaea.

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Quinacrine Fluorescence Patterns of Human *D* Group Chromosomes

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Quinacrine fluorescence patterns have been used to identify ten different numerical and structural abnormalities of human chromosomes 13, 14 and 15. There are several advantages of this method over autoradiographic and morphological methods.

shown that all the chromosomes in the human complement can be paired by these patterns, which can be analysed most accurately by a quantitative spectrofluorimetric method. Visual analysis of fluorescence patterns has been used to identify the *Y* chromosome³⁻⁵ and the *G* group chromosomes^{6,7}.

We report here the usefulness of visual analysis of quinacrine fluorescence patterns for identifying chromosomes of the *D* group, for characterizing numerical and structural abnormalities of these chromosomes and for identifying the origin of structurally abnormal chromosomes.

QUINACRINE and its derivative, quinacrine mustard, bind to specific regions of chromosomes, producing characteristic and reproducible patterns of fluorescence. Caspersson *et al.*^{1,2} have

Preparation of Cells

Studies were carried out on eighteen individuals with *D* group chromosome abnormalities (Table 1). Standard metaphase

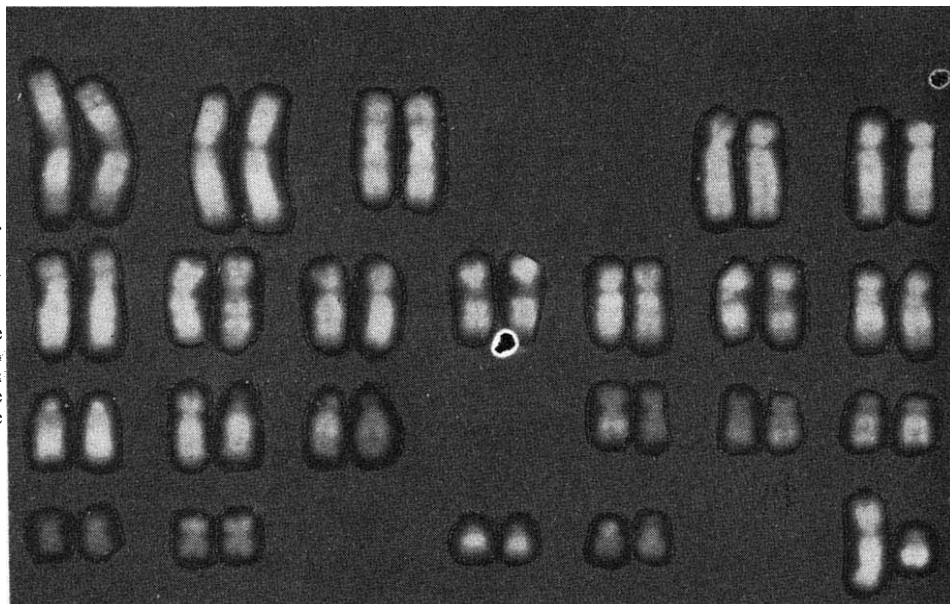
Table 1 Identification of Cases

Case No.*	Fig. No.	No. of cells	By fluorescence†	Karyotype	Does autoradiography agree?
MK260770	2a	37	47,XY,13+		Yes
BG241070	3a	13	47,XX,13+		Yes
LK201168	2b	42	46,XX,13q-		Yes
KS130342	3b	17	46,XX,14p-		Yes
MD041126	3c	10	47,XXY,14p-		—
CR190558	3d	30	46,XY,15p-		Yes
ML160670	3e, f	14	46,XY,13-,t(13q13q)+/46,XY,13r		—
RD040457	3g	18	45,XY,13-,14-,t(13q14q)+		—
EO260746	3h	8	46,XX,13-,14-,21+,t(13q14q)+		—
BB111270	3i	23	47,XY,(14q-)+		—
LH280298	3j	16	45,XX,14-,21-,t(14q21q)+		—
OH280826	3k	20	46,XY,14-,t(14q21q)+		Yes
BS170646	3l	8	45,XX,14-,21-,t(14q21q)+		—
RS040969	3m	6	46,XY,14-,t(14q21q)+		—
NK070239	3n	11	46,XY,14-,t(14q21q)+		No
NE050830	3o	19	46,XX,14-,t(14q21q)+		Yes
TM240219	1	19	46,XY,14-,t(14q21q)+		Yes
EL080714	3p	9	46,XY,5p-,14-,t(14q;5p)+		—

* Initials, birthdate (day, month, year)¹³.

† (+) Additional; (-) absent or deleted; (p) short arm; (q) long arm; (r) ring; t(13q14q) centric fusion; t(14q;5p) reciprocal translocation¹³.

Fig. 1 A 46,XY,14-,t(14q21q)+ karyotype prepared from a single print of a metaphase cell from case TM240219, a male with trisomy 21 (Down's) syndrome. The placement of chromosome pairs is that used by Caspersson². Note the close similarity in fluorescence patterns of each pair of autosomes, and between each arm of the translocation chromosome and the corresponding arm of the chromosome from which it was delivered.



chromosome preparations were made from cultured leucocytes stimulated with phytohaemagglutinin (PHA), sometimes with the addition of tritiated thymidine⁸. Freshly prepared slides of air-dried metaphase spreads were treated with a 0.5 or 1.0% solution of quinacrine hydrochloride ('Atebrin', Gurr) for 5–7 min, rinsed for 3 min in water, and then rinsed and mounted in Tris-maleate buffer, pH 5.6.

Cells were examined with a Leitz 'Ortholux' microscope by transmitted ultraviolet light from an HBO 200 W mercury vapour lamp with a 1.5 mm BG 12 exciter filter, a well-oiled darkfield condenser (D1.20), a K530 or K510 barrier filter, and a $\times 90$ apochromatic oil immersion objective. Well spread metaphase cells were photographed on Kodak 'Panatomic-X' film (exposures 20–30 s).

Full karyotypes were prepared on 59 of the 320 metaphase figures photographed, and partial karyotypes on many others. In some cases, the coverslip was removed after the fluorescence metaphase figures had been photographed and the cells were stained with Giemsa and photographed again. A third photograph was taken of each tritium-labelled metaphase after application of Kodak AR-10 stripping film to the slides, exposure in the dark for 7–10 days, and development in D-19. The autoradiographic patterns of the *D* group chromosomes were observed in ten cells from MK, seventeen from BG and forty-two from LK.

Trisomy 13 Syndrome

In the karyotyped cells, all the normal chromosomes could be separated into pairs on the basis of their distinctive patterns of fluorescence (Fig. 1). In cells from two infants with the clinical features of the trisomy 13 syndrome, there were seven *D* group chromosomes and autoradiography showed three chromosomes 13. Fluorescent studies showed three chromosomes which fluoresced brightly over the distal half of the long arm (Figs. 2a and 3a). In those cells in which three chromosomes 13 could be identified autoradiographically, the same three showed the typical fluorescence pattern identified with chromosome 13.

One previously unstudied patient with the typical clinical features of the 13q-deletion syndrome⁹ had a 13q-chromosome which was identified by both fluorescence and autoradiography (Fig. 2b). The abnormal chromosome appeared shorter than its homologue, but had the bright fluorescent banding and heavy terminal labelling of chromosome 13. Because the patterns characteristic of the distal half of the long arm were still present in the abnormal chromosome, an interstitial segment of non-late-replicating, non-highly-fluorescent chromatin was probably deleted from the proximal part of the long arm.

Three of the patients studied had a *D* group chromosome from which the short arm was deleted. In one patient the deleted chromosome was previously identified autoradiographically as chromosome 14¹⁰ (case 2). This deleted 14p-chromosome fluoresced brightly over the proximal half and in a narrow band near, but not at, the distal end of the long arm, confirming this long arm pattern as characteristic of chromosome 14 (Fig. 3b). A similar marker chromosome was identified by fluorescence as a 14p- in a second patient (Fig. 3c). Finally, in a patient with a 15p- chromosome (identified by autoradiography¹⁰ (case 3)) the deleted chromosome was one of the pair with a fluorescent band in only the proximal half of the long arm, confirming the identification of this long arm pattern as that of chromosome 15 (Fig. 3d).

One patient who had seven *D* group chromosomes did not seem to have the clinical features of the trisomy 13 syndrome.

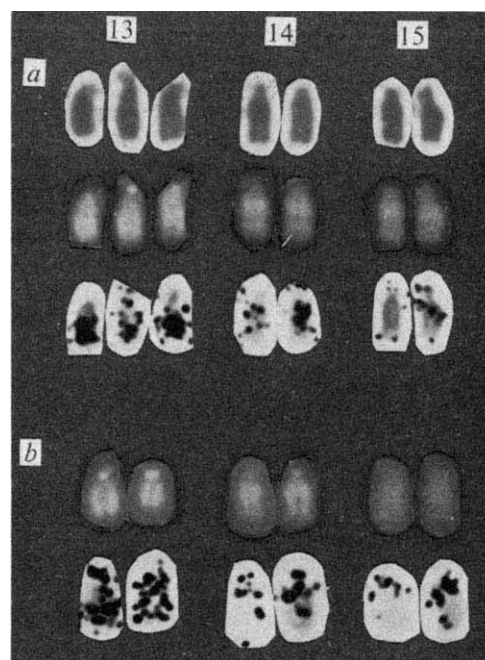


Fig. 2 *D* group chromosomes from individuals with: (a) trisomy-13; and (b) a 13q-deletion, observed using Giemsa, quinacrine fluorescence and autoradiography after growth in the presence of tritiated thymidine to show terminal DNA synthesis. Chromosomes were paired solely by their quinacrine fluorescence patterns.

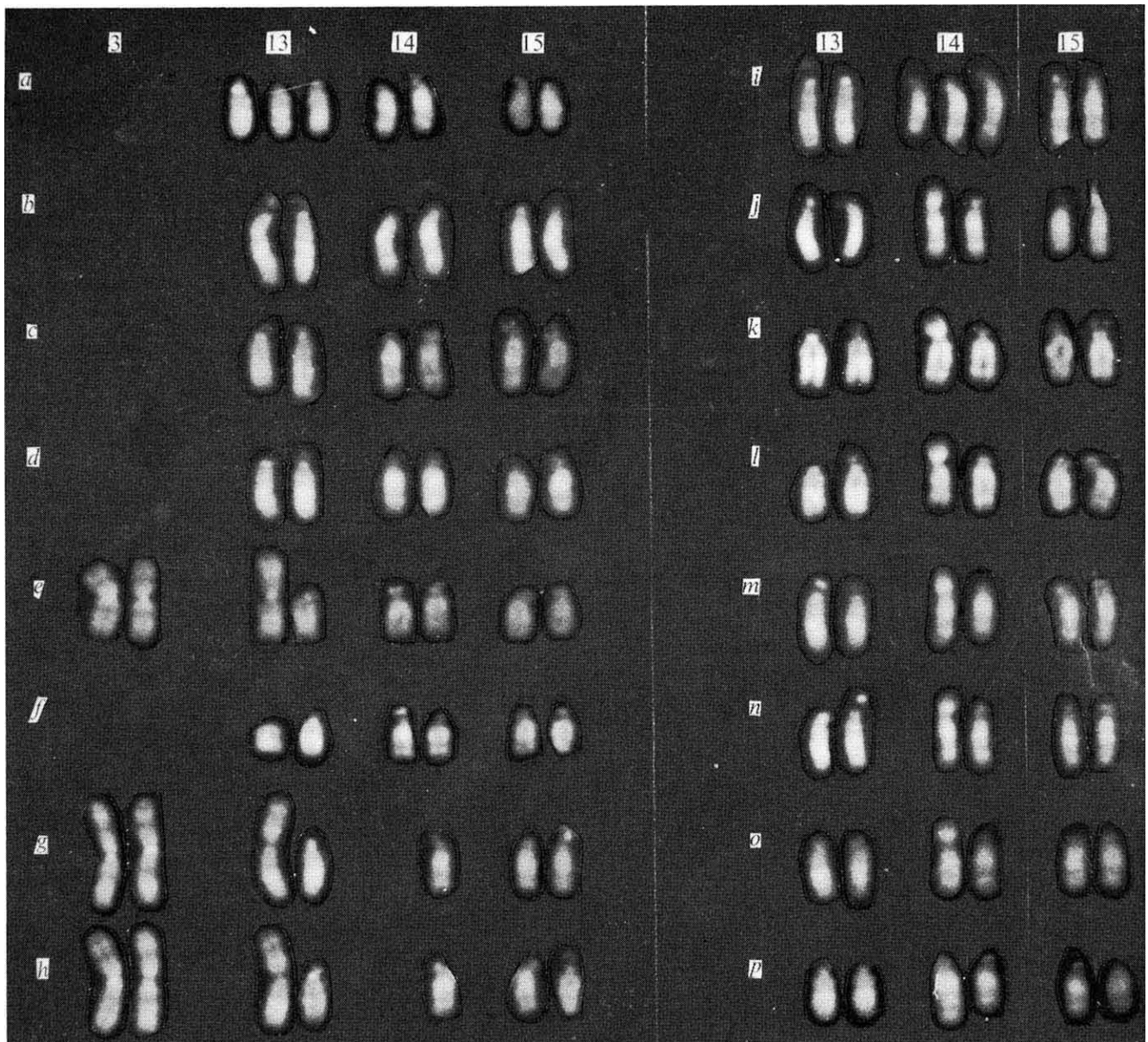


Fig. 3 Partial fluorescent karyotypes from fifteen individuals: (a) trisomy-13; (b and c) $14p-$; (d) $15p-$; (e) $t(13q13q)$; (f) 13 ring, same case as e; (g and h) $t(13q14q)$; (i) partial trisomy-($14q-$); (j-o) $t(14q21q)$, j mother of k, l mother of m; (p) $t(14q; 5p)$. The abnormal chromosome is the left member of the relevant pair.

The additional chromosome in this case was not identical to any D group chromosome (Fig. 3i), but appeared similar to a shortened chromosome 14. Studies of cells from the mother of this patient showed that she had a reciprocal translocation involving chromosomes 9 and 14. The son is therefore trisomic for most of chromosome 14.

Translocation Chromosomes

Translocation chromosomes retain the distinctive quinacrine fluorescence patterns of the chromosomes from which they are derived. This is clearly visible in translocation chromosomes produced by the, presumably, centric fusion of two D or of one D and one G group chromosomes. Fig. 3 shows translocation chromosomes from one individual with a $t(13q13q)$, two with a $t(13q14q)$, and six (four previously studied by autoradiography¹¹) with a $t(14q21q)$.

The first type of translocation, $t(13q13q)$ (Fig. 3e), was characterized by a symmetrical distribution of the fluorescence

pattern matching that found in the normal chromosome 13. Furthermore, a small bright band was present in the centromeric region of the translocation chromosome, possibly indicating retention of the short arm of one of the chromosomes. Chromosome 3, which is similar in length to the translocated chromosome, was easily distinguished from the translocation chromosome, as shown in Fig. 3e. A minority of cells in this case contained a ring chromosome instead of the translocation chromosome (Fig. 3f).

In the second type of translocation, $t(13q14q)$ (Figs. 3g and h), the fluorescence of one arm matched that of chromosome 13 and that of the other arm matched chromosome 14. In each case, only one chromosome 13 and one 14 were present and the translocated chromosome could be distinguished from chromosome 3.

In the third type of translocation, $t(14q21q)$ (Figs. 3j-o), the short arm had a single bright band matching that of chromosome 21, while the long arm matched chromosome 14. The resultant pattern of fluorescence is unlike that of any C group chromosome.

Cells from a carrier of a balanced reciprocal translocation, $t(5p-; Dq+)$, contained one *D* group chromosome which was slightly longer than the others. Measurement of the abnormal chromosome led us to suggest that chromosome 14 or 15 was involved¹². Fluorescent studies showed that the long chromosome had the pattern of chromosome 14 plus an additional bright band at the distal end of the long arm (Fig. 3*p*), presumably derived from the short arm of chromosome 5.

The consistency of finding pairs of chromosomes with each of the three distinct *D* group fluorescent patterns was checked in about 300 cells from twenty-four individuals, three with normal and twenty-one with abnormal karyotypes, but normal *D* group chromosomes. A pair of chromosomes with the fluorescent pattern characteristic of number 13 could be identified in almost every cell from each case. The frequency with which chromosome 14 could be distinguished from chromosome 15 depended partly on the quality of the preparation. The minor band near the distal end of chromosome 14 was clear in only about 50% of the cells; when the minor band was not present, chromosome 14 appeared similar to chromosome 15.

Distinguishing *D* Group Pairs

The human *D* group contains three pairs of homologous chromosomes. The first method used to differentiate between them, based on morphology¹³, is inadequate because of their small differences in length and arm ratio, and their variable number and size of satellites. A second method, autoradiographic analysis of terminal DNA synthetic patterns, enables much more accurate identification of chromosomes within this group to be made, whether by qualitative pattern analysis¹⁴ or quantitative analysis of grain counts¹⁵. Even better discrimination between chromosomes is possible by combining the two approaches, that is, by considering the location as well as the number of grains over each *D* group chromosome¹⁶.

The autoradiographic method has several important limitations: it is time consuming; the study must be planned before the culture is harvested, that is, usually before the karyotype is known; and errors in identification can occur when a small sample of cells is scored, because of the statistical nature of radioactive decay and its autoradiographic analysis¹⁷.

Quinacrine or quinacrine mustard fluorescence patterns² provide by far the best method available so far for identifying human chromosomes. This is the only method which permits identification of specific chromosomes within the *C*, *F*, and *G* groups, and rapid identification of both normal and translocation chromosomes. The quinacrine fluorescence technique can be used with routine chromosome preparations and does not require prior planning, as the chromosomes can be observed first on a stained test slide. Furthermore, this technique, unlike autoradiography, does not depend on probability and even a few cells may be sufficient for a reliable identification of a chromosomal abnormality.

Chromosome 13 is the easiest member of the *D* group to identify. As well as the bright fluorescent band over the long arm, there is often a very bright centromeric region¹⁸ (Figs. 1, 2 and 3) or satellite (Figs. 3*b*, *j*, *m* and *n*). When only one chromosome of the pair has such a marker it serves to distinguish between the homologous chromosomes. The ease of identification of chromosome 13 is emphasized by our results in the 13*q*- case, in which the deletion could be shown to be interstitial, involving the loss of a segment near the centromere.

Chromosomes 14 and 15 are not as easily distinguished from one another by their fluorescence patterns as they are from chromosome 13, and a very bright centromere or satellite is present less often (Figs. 3*c*, *e*, *f*, *g* and *i*). Nevertheless, each chromosome can be clearly identified by this technique in a sample of only a few cells.

Techniques for Future Studies

One of the most significant results of this study was the finding that translocation chromosomes retain the distinctive

quinacrine fluorescence patterns of the chromosomes from which they are derived. This was true for each of the centric fusion translocation chromosomes: $t(13q13q)$, $t(13q14q)$ and $t(14q21q)$. It was possible to show that a translocation which we had previously identified¹² by autoradiography as a $t(15qGq)$ is in reality a $t(14q21q)$ (NK). Such translocation chromosomes are morphologically similar to some of the *C* group chromosomes, and cannot be distinguished by autoradiography (being identified in this technique indirectly by demonstrating a missing labelling pattern among the remaining *D* group chromosomes). Using fluorescence, however, the translocation chromosome can be identified directly.

The retention of the distinctive pattern of fluorescence of each chromosome segment facilitates the identification of other abnormal chromosomes. The $t(14q;5p)$ chromosome, which was difficult to see in Giemsa-stained karyotypes, is easily recognized in fluorescent cells. Because this chromosome was present in a normal individual who has a reciprocal $(14q;5p)$ translocation, the source of the additional material was clearly the deleted chromosome 5. The identity of the abnormal chromosome in case BB111270 (Fig. 3*i*), however, remained unclear even in the fluorescent karyotypes. Study of the mother's chromosomes revealed that she had a reciprocal translocation between chromosomes 9 and 14, permitting identification of the abnormal chromosome in the affected individual as a 14*q*-.

The quinacrine fluorescence technique will clearly be the method of choice in future studies on suspected chromosomal abnormalities, although the interpretation of some of the findings will still depend on information derived from parental karyotypes. The advantages of the quinacrine fluorescence method suggest that this technique would be ideally suited for prenatal diagnosis of chromosomal disorders. In preliminary studies (unpublished) we have found no difference in the fluorescence patterns of metaphase chromosomes from cultured amniotic fluid cells, fibroblasts or leucocytes.

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Microsurgical Studies on Human Cells and Cloning of HeLa Cells

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This article describes the equipment and microsurgical procedures requisite to dissecting, injecting or otherwise altering human cells of normal or malignant origin. These procedures, amenable to automation, are also requisite to the isolation and cloning of solitary, operated HeLa cells.

THE techniques of microsurgery on single cells¹ have been applied to a variety of cell types²⁻⁸, but not often to human cells in culture⁹⁻¹². What follows is a description of a microsurgical methodology developed to study human cells of normal or malignant origin and which has been used to dissect, inject and otherwise to alter interphase or mitotic cells¹³.

A cell on which microsurgery has been performed may conveniently be studied by isolating it from its neighbours and transferring it, as a solitary cell, to a microenvironment in which it can survive with or without cell division, give rise to a clone and ultimately a clonally derived cell line. The cell may survive without dividing for three reasons: (a) it cannot divide even before the operation; (b) it can divide after the operation but the microenvironment is not suitable; or (c) it can divide before but not after the operation. The first two reasons depend on cell type, the third on the operation. We have developed a method for cloning solitary, operated HeLa cells which exploits their capacity to divide and demonstrates that operations do not interfere with cell proliferation.

All microtools are finished in a de Fonbrune microforge (Aloe) equipped with a monocular body tube from a compound microscope with a $\times 3.5$ and a $\times 50$ UMK Leitz objective. A U-shaped, platinum-iridium filament is heated to bend and draw the microtips. Microforge operations are viewed through a $\times 10$ or a $\times 20$ ocular at magnifications ranging from $\times 35$ to $\times 1,000$.

The modified Leitz unit is shown in Fig. 1. Fig. 2 is a closer view of the microscope stage showing the microtools in their

holders and extending into the microsurgical chamber, in which all microoperations are carried out. Four strips (5 mm $\times$ 25 mm $\times$ 1 mm) are cut from a glass slide and are cemented on the base, which is a whole glass slide (2 inch $\times$ 2 inch $\times$ 1 mm). They are positioned to support the corners of an 18 mm square coverslip which roofs the chamber (Fig. 2). There is a horizontal clearance for the microtools of at least 10 mm between adjacent supports. The vertical clearance is 1 mm within the chamber. The chamber is 2 mm high to facilitate the use of high magnification ($\times 1,875$).

The microneedles are made from 1 mm 'Pyrex' or borosilicate glass rod drawn out⁴ to form a thin (~ 0.3 mm) shaft, ~ 50 mm long. The vertical bend is made (b, Fig. 3) to provide clearance between the microtool holder and the microscope stage (Fig. 2). The thinner shaft is trimmed to 30 mm and the end is hooked to carry a weight.

The shaft of the needle, loaded with 1 g, is drawn out at an angle of 45° in the heated microforge. A microtip is formed at the surface of a molten glass bead and the microshaft (e) is bent against the filament to produce the finished needle (Fig. 3). The microshaft (e) is slightly angled ($\sim 5^\circ$) to elevate the microtip.

Micropipettes are made, just before injection, from 1 mm, thin walled, glass capillaries (Aloe) coated with 'Desicote' (Beckman), and drawn like needles except that the large lumen is maintained. The thinner portion of the capillary is trimmed and hooked, and the pipette is connected to a silicone oil reservoir and mounted in the microforge. The thinner portion is drawn to a fine capillary at a 45° angle by heating, using a 250 mg load. The pipette orifice is formed at the surface of the glass bead and may be bevelled⁸.

If fluids other than silicone oil are to be injected, the unfinished pipette is backloaded with the solution and then connected to the silicone oil reservoir. The micropipette is then finished before the solution is forced to the tip, with care to avoid heating the solution in the capillary. Micropipettes and microneedles, which have the same dimensions, are broken in antiseptic solution after each day's surgery.

HeLa, ERK, and HEL (human embryonic lung) cells are grown in coverslip microcultures for microsurgery in Dulbecco's medium¹⁴. Two sterile, 18 mm square coverslips are inserted into each sterile, plastic Petri dish, which is inoculated with

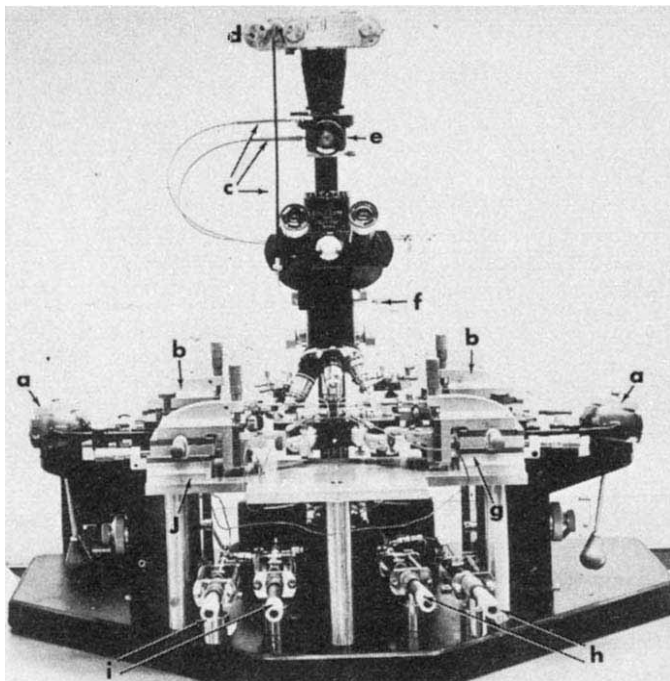


Fig. 1 The instrument shown is comprised of a Laborlux II microscope (Leitz) equipped with a tri-ocular head so that operations can be viewed with $\times 15$ wide-field oculars (Galileo Co.) and photographs can be taken with the Micro-Ibso attachment (*c, d, e*) by shifting the prism (slide *f*). The objective turret holds $\times 2.5$ Zeiss bright field, $\times 10$ Leitz phase contrast, $\times 100$ Neofluor Zeiss objectives, and a Leitz scribe. The photographic attachment is shown with the Leica camera back (*d*), viewer (*e*), and cable releases (*c*). The black cable release controls the camera shutter (*d*) which is normally left open while the frame is exposed and the exposure is timed with the shutter of the Micro-Ibso attachment. The camera shutter is then closed and the film is advanced. The metallic cable releases (*c*) control the prism and shutter of the attachment. Microscope illumination is of the Kohler type using an Osram source. The intensity of the light is adjusted by a variable transformer (not shown). The microscope is attached to the baseplate which is mounted on a shock-absorbing mounting (part of top plate is visible). Six micropositioners are attached. Two Leitz micropositioners (*a*) are fastened to the baseplate. Four Line Tool Co. micropositioners (*b, j, g*) are attached to aluminium platforms which raise the positioners to the appropriate height. Each micropositioner is equipped with a Leitz single or double microinstrument clamp. The injection systems (*h, i*) are two pairs of interconnected microinjection units (Aloe Co.). The micro-meter heads seen are used to control and maintain pressure. The controls are mounted contralaterally so that unit (*i*) controls the pipette mounted in positioner (*g*). The syringe reservoirs (not shown) are coupled to the pipette holders with rigid, thin-walled, stainless-steel, capillary tubing.

5 ml. of cell suspension. Cells attach and grow on the coverslips during incubation at 37°C in a humidified atmosphere containing 5% carbon dioxide. Such cultures can be used on successive days.

Microsurgery

To align the microtools, a plain glass slide (2 inch $\times$ 3 inch $\times$ 1 mm) is clamped on the microscope stage to protect the condenser and to provide a reflecting surface. Each micro-needle in its holder is advanced in the micropositioner clamp until visible in the field at $\times 56.2$, and then manoeuvred until the bend (*b*, Fig. 3) is perpendicular to the slide surface and the shaft (*c*, Fig. 3) is parallel with its image reflected from the slide surface, so that the microtip can reach the coverslip with the chamber in place. The micropositioner clamp is then tightened.

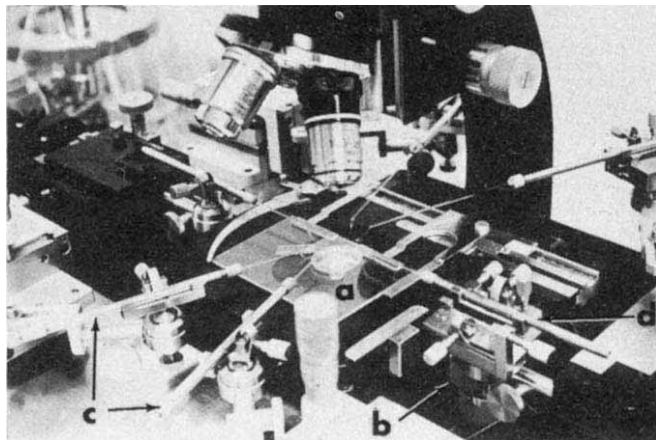


Fig. 2 The orientation of the microinstruments and the microsurgical chamber is shown on the microscope stage. The microsurgical chamber (*a*) is roofed with a blank coverslip and the microtools extend into the chamber through the openings on the four sides. The coaxial substage control (*b*) is visible as are the stage verniers. The micropipette holder (*d*) is mounted in the double microinstrument clamp.

Pipettes are aligned in the same way. If rigid tubing connects the pipette holder to the syringe reservoir, the bend (*b*, Fig. 3) must be aligned before the holder is inserted in the clamp (*c*, Fig. 2). Pressure in the injection system should be equilibrated at this time.

Properly positioned, the six microtools form a hexagon centred about the optical axis at $\times 56.2$. The hexagon is slightly larger than the optical field at high magnification ($\times 1,875$).

The optics remain adjusted for phase contrast viewing at $\times 1,875$. At low magnifications ($\times 56.2$ and $\times 187.5$) objects appear in dark field. Objectives are positioned by raising the body tube, selecting the objective, and then lowering the body tube, to avoid striking the vertical bend (*b*, Fig. 3) of the microtools which extends above the chamber.

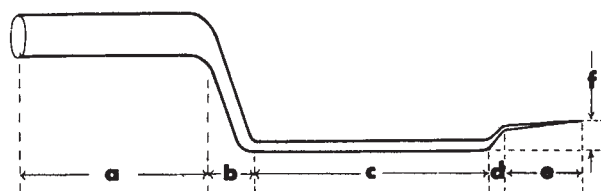


Fig. 3 The shape and relative dimensions of the microtools are diagrammed in this figure. The total length of the microtool depends on the distance between the micropositioner and the optical axis of the microscope. Any variation in total length is adjusted by varying the length of (*a*). The microtool is diagrammed from a side view, and the height (*f*) should not exceed 0.5 mm. In top view the microtool would appear as a straight glass rod of decreasing thickness.

The tools are raised about 3 mm and the plain slide is removed. A sterile chamber (without a coverslip) is inserted into the stage clamp, centred with the optical axis, and the needles are lowered among the glass supports until their shafts (*c*, Fig. 3) just clear the floor of the chamber. A plain, sterile coverslip is anchored to the supports which have been coated with silicone grease.

A final check is made to see that the microtip of each tool

can reach the bottom surface of the coverslip at $\times 187.5$. Each is raised to the coverslip and then lowered to its previous position.

The plain coverslip is removed and a coverslip microculture is inverted and anchored on the chamber, which is filled with growth medium. The openings on the four sides are sealed with a thin layer of silicone oil saturated with growth medium at 37°C . This retards evaporation and helps to maintain sterility. The exposed surface of the coverslip is wiped, cleaned with distilled water or lens cleaner, and dried with cotton-tipped applicators.

A Leitz scribe (Fig. 1) is lowered to the surface of the coverslip and a circle, 4 mm in diameter, is etched on the coverslip for cell isolation. A drop of immersion oil is put on the coverslip for $\times 1,875$ magnification which does not interfere with viewing at lower magnifications.

A cell is selected at $\times 187.5$, then located and centred at $\times 1,875$, its position is recorded, and the cell is identified at $\times 187.5$. The microtools are positioned under the cell but not raised yet.

The cell is focused at $\times 1,875$ and the microtools are raised until they are just beneath the cell. The viewer must know the position of each tool with respect to the cell and to the other tools at all times. A tool not in immediate use should be lowered and out of the $\times 1,875$ field to prevent optical aberration or touching other cells or tools. The vertical position of each tool must be kept in mind because the shaft (c, Fig. 3) may touch the floor of the chamber and bend, causing an upward deflexion of the microtip even though the holder is being lowered. Conversely, if the needle is raised too much, the microshaft (e) will cut through cells and bend against the coverslip causing a downward deflexion of the tip and breaking the microshaft (e).

A needle is brought into the cell at some selected focal plane within the cell at $\times 1,875$. To do so, the needle is positioned so that it lies just outside the cell in the selected focal plane. By focusing the cell and manoeuvring the needle, the microtip can be positioned precisely. The needle is withdrawn from the cell in a horizontal plane with concurrent vertical lowering in small repetitive steps to avoid tearing the cell.

Silicone oil is used as the injection fluid to determine the required size of the pipette orifice and to develop the injection procedure¹³.

Positioning pipettes for injecting cells is similar to positioning needles. The only difference is that fine pipettes ($0.5\text{--}1\text{ }\mu\text{m}$ in diameter) are displaced as the pressure in the injection system is increased to expel fluid, but if pressure is increased slowly and the intracellular position of the pipette is maintained, the site of injection is localized with precision. One or more injections may be made. The pressure is equilibrated making compensatory adjustments to maintain pipette position and then the pipette is withdrawn carefully.

The injection system must be free of air and air-tight, and should contain one mechanical coupling which disconnects if the pressure becomes too high. Even in large pipettes ($2\text{--}3\text{ }\mu\text{m}$), used for transplantation or chromosome extraction, the pressure must be monitored to avoid aspirating fluid from the chamber into the pipette.

Operations on cells, photographed just before and after surgery, are recorded on 'Panatomic-X' 35 mm film using the Leitz attachment (Fig. 1), and the time is recorded. Microtools should be out of the cell when photographs are taken or when film is advanced.

Cloning Solitary Cells

An operated cell can be isolated by selectively killing the surrounding cells and allowing sufficient time for the connexions between the operated cell and its killed neighbours to be retracted. Then, with one or more needles, the neighbouring cells can be removed from the coverslip and allowed to fall to the chamber floor. The operations should be viewed at $\times 1,875$.

Using lower magnification ($\times 187.5$) the microshaft (e, Fig. 3) of a needle is pressed against the coverslip to sweep away unwanted cells from within the etched circle and a short distance beyond.

An operated interphase cell can be isolated at any time; an operated mitotic cell is best isolated when mitosis is complete and at least one of the daughters should be spread on the coverslip to avoid losing the cells later. Needles and pipettes are carefully withdrawn from the chamber, which is then incubated or transferred to a sterile hood in which the following procedures are carried out as quickly as possible in sterile conditions.

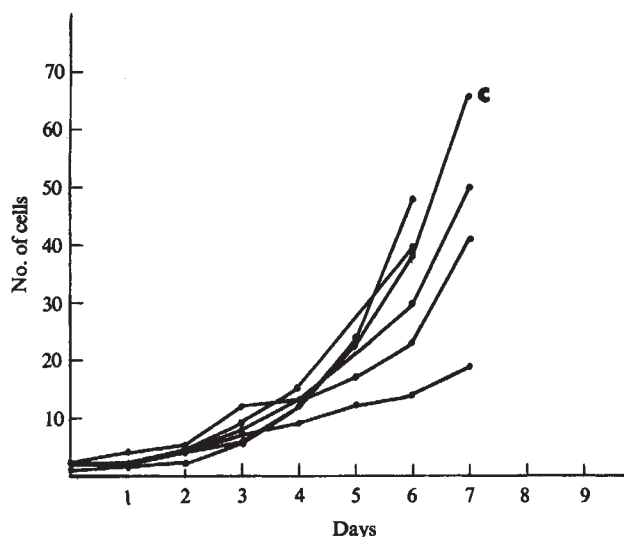


Fig. 4 The initial segments of growth curves of clonal aggregates are shown. These aggregates are the progeny of cells in which chromosomes were manipulated, DNA and sodium chloride solutions were injected, or of cells subjected to nuclear micropuncture or manipulation during mitosis. Growth curve (c) shows the early proliferation data for an unoperated control cell for comparison with the other curves; this clone is not listed in Table 1.

The upper, exposed surface of the coverslip roofing the chamber is cleaned of immersion oil, wiped with ethanol and dried using cotton-tipped applicators. The coverslip is removed and placed, cell side up, in a plastic Petri dish containing about 7 ml. of growth medium supplemented with 20% serum. The dish is transferred to the stage of a stereoscopic dissecting microscope (Leitz) for the following procedures.

With the cell within the etched circle on the coverslip, the dish is opened and lines are etched, with a diamond scribe, from the edge of the circle to the edges of the coverslip. Gentle pressure frees the circular fragment from the rest of the coverslip.

The fragment is placed on the floor of another Petri dish with 5 ml. of growth medium so that only the operated cell is attached. Then it is gently swirled to wash it and the fragment is transferred to a partially assembled SMC (Sykes-Moore chamber)^{15,16} within a glass Petri dish.

After the bottom coverslip has been inserted in the metal base of the SMC and the silicone rubber O-ring spacer is in position the fragment is centred on the coverslip, and the top coverslip and metal top are placed and partially tightened. The SMC in the dish is transferred to a sterile glove box for the final tightening and filling of the SMC^{15,16}.

It is advantageous to use culture medium which is refiltered through a Swinney filter adaptor (GS filter; 'Millipore') and to use No. 26 needles. Since the fragment lies free on the chamber floor, it is necessary to fill the chamber, to change medium¹⁶,

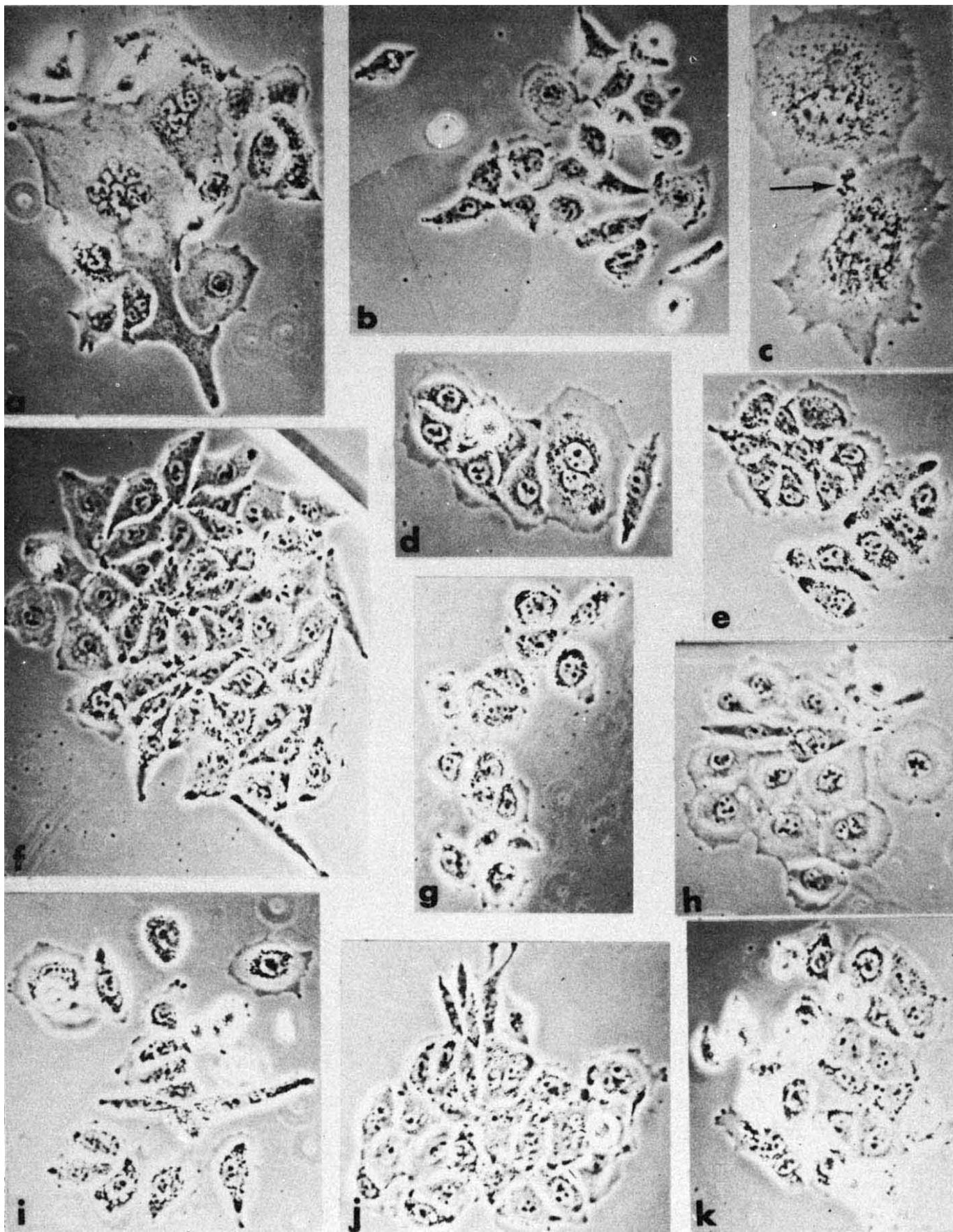


Fig. 5 The photographs show young clonal aggregates from cells injected with HeLa-DNA (*a, b, d, e, f, i*) and sodium chloride (*g, h, j, and k*). These show the variation in the morphology of the aggregates and of the cells within each aggregate as they were checked and photographed daily. *c*, Two daughter cells recently separated and with trapped chromosomes between them (arrow) to show the detailed cell features which can be checked ($\times 470$). All other photographs are $\times 187.5$.

Table 1 Clones and Clonally Derived Lines from Solitary, Operated HeLa Cells

Operation, cell and date	Clone	Clonally derived line (length of time carried)
Intracytoplasmic microinjection:		
4 2- 8-67	+	+ (2 years)
Intracellular chromosome manipulation:		
2 8- 3-67	+	+
1 8- 8-67	+	- *
2 8- 8-67	+	+
3 6-14-68	+	+
6 7- 2-68	+	-
4 12-12-68	+	-
Nuclear micropuncture:		
2 4-29-69	+	-
DNA injection into nucleus or among chromosomes:		
2 7-23-68	+	+ (1 year)
4 7-23-68	+	+ (1 year)
7 7-24-68	+	-
1 8- 6-68	+	+ (1 year)
5 8- 6-68	+	-
6 8- 9-68	+	+ (1 year)
NaCl (0.15 M) injection into nucleus or among chromosomes:		
1 8-27-68	+	-
1 10-10-68	+	+ (1 year)
6 10-10-68	+	+ (1 year)
Surface effects of oil during mitosis:		
3 6- 8-68	+	+
1 6-26-68	+	+
2 8-28-68	+	-
Golgi manipulation during mitosis:		
4 6- 8-68	+	-
Total	21	11

* Not carried for any reason other than failure to proliferate beyond the minimum of 50 cells.

and to transport the SMC with care. It is placed in a metal carrier slide, and then the cell is examined with an inverted microscope (Zeiss) with phase contrast optics. The SMC is replaced in the glass Petri dish and incubated at 37° C in a humidified atmosphere of 5% CO₂-95% air. The clonal aggregate is checked daily and defined as a clone when the number of cells reaches 50 or more. After 3-4 weeks, there is a macroscopically visible clone which can be transferred to a culture flask using sterile technique in a sterile hood.

Clone Transfer

The SMC surface is cleaned with ethanol. The metal top is removed and the coverslips with the O-ring spacer are removed as a unit and immersed in growth medium (10% serum) in a Petri dish. The top coverslip and O-ring spacer are removed. The fragment bearing the clone is transferred to a dry, plastic Petri dish. Cotton-plugged Pasteur pipettes are used to transfer the small volumes required to just cover the fragment. Two washes with phosphate-buffered saline (calcium and magnesium deficient) are followed by trypsinization. The cells are suspended in 5 ml. of growth medium and inoculated into about 15 ml. of growth medium in a culture flask, in which the air is replaced with a 5% CO₂-95% air mixture before closure and incubation at 37° C.

Solitary, operated HeLa cells divide within 48 h in the SMC. Proliferation rates from the first few mitoses are illustrated in Fig. 4, and Fig. 5 shows some young clonal aggregates from solitary, injected HeLa cells.

Table 1 lists the cells from which clones of at least fifty cells were obtained, grouped according to the operation done. Those which formed macroscopically visible clones were transferred, and carried as clonally derived cell lines, are also indicated in Table 1. The operations done include intracytoplasmic injection, chromosome manipulation¹⁷, nuclear micropuncture (needle only), and microinjection of HeLa DNA in sodium chloride or sodium chloride solution into the nucleus of intact interphase cells or among chromosomes of intact mitotic cells (our unpublished results).

Advantages of the Techniques

Munro¹² and Munro and Daniel¹⁸ studied the progeny of small numbers of unoperated HeLa or cut Chinese hamster cells left *in situ* in special chambers. The preparation¹² microcultures reduced the total number of cells and the number of mitotic cells available for microsurgery. Moreover, if only three or four cells are isolated and left *in situ* on the coverslip, it is difficult to separate and to identify unequivocally the progeny of the individual cells.

Our procedures eliminate the preselection of cells, insure unequivocal identification of the progeny of an individual cell, and eliminate the effects of the metabolism of other clonal aggregates in the environment. Clones and clonally derived cell lines from solitary HeLa cells demonstrate that operations on HeLa cells at different times in the cell cycle do not interfere with mitosis nor preclude prolonged proliferation.

The basic microsurgical procedures which are vital to cell isolation and cloning and which are amenable to automation can no doubt be applied to other types of mammalian somatic cells. The cloning method developed for HeLa cells may be modified to provide the option for either short or long term study of the effects of operations on other types of cells.

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Chromosome Displacement in and Extraction from Human Cells at Different Mitotic Stages

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Chromosomes of HeLa, ERK, and human embryonic lung cells have been manipulated at different mitotic stages. A method for releasing chromosomes from metaphase human embryonic lung cells has been developed and seven HeLa cell lines have been established from individual mitotic cells isolated and cloned after manipulation.

THE micromanipulation of chromosomes of living human cells *in vitro* is a potential means of obtaining transplantable genetic material, a method for altering chromosome numbers, and a means of obtaining more detailed analysis of chromosomes¹⁻⁵. In the course of an investigation⁵ of the feasibility of manipulating and isolating chromosomes from cells of normal and malignant origin without chemical pretreatment, it was found that there were differences in the ease with which chromosomes could be manipulated or released at different mitotic stages. And there were differences in the response of different cell types to physical methods developed for obtaining chromosomes from normal cells of human embryonic lung (HEL) as compared with HeLa and ERK (a subline of HeLa) cells.

The methods of cell culture and the basic equipment and preliminary procedures for microsurgery have been described⁶

already. Mitotic cells within the cell layer attached to the coverslip used to roof the microsurgical chamber are subjected to microsurgery. Because mitotic cells constantly change in appearance, neighbouring interphase cells are used as markers.

Clones were established⁶ and then carried as regular cell lines designated by a code derived from the parent line from which the microcultures were prepared, the cell number, and the date of operation. Thus, clone HeLa-C-28367 represents a line derived from the second cell subjected to surgery on August 3, 1967.

The chromosomes visible in prophase cells can be probed with extremely fine needles and division will continue. HeLa, ERK, and HEL cells (Table 1) have been studied and respond in a similar manner. Attempts to release the chromosomes when the nuclear membrane is still clearly discernible have failed. The cell in prophase is more susceptible to injury than at later stages. HeLa cells have been manipulated, and it has been possible to probe the chromocentre of a prophase cell and to obtain a clone from that cell (Table 2).

Chromosomes in HeLa, ERK, and HEL cells can be displaced readily with the tip of a very fine needle and division will continue. As an individual chromosome is moved, adjacent chromosomes are displaced to a lesser extent but moved in the same direction (Fig. 1). As soon as the chromosome is freed

Table 1 Cells Manipulated at Different Mitotic Stages

Mitotic stage	HEL	Cell type ERK	HeLa	No. of cells
Prophase	19	15	23	57
Prometaphase	90	21	20	131
Metaphase	231	50	46	327
Anaphase	60	18	28	106
Telophase	33	46	52	131
Total	433	150	169	752

Table 2 HeLa Lines Clonally Derived from Single Mitotic Cells subjected to Microsurgery

Cell line	Microoperation
HeLa-C-28367	Chromosomes displaced during prometaphase
HeLa-C-28867	Chromosomes displaced during prometaphase
HeLa-C-2'102567	Chromosomes displaced during metaphase
HeLa-C-3101867	Chromosomes displaced during metaphase
HeLa-C-36868	Telophase cells capped with silicone oil
HeLa-C-361468	Prophase nucleolar mass probed
HeLa-C-162668	Telophase cells capped with silicone oil

After each of these microoperations, the mitotic cell was isolated as a solitary mitotic cell and a clone was obtained. After the clonal aggregate was visible macroscopically, it was transferred⁶, carried for several passages, and the cells were frozen.

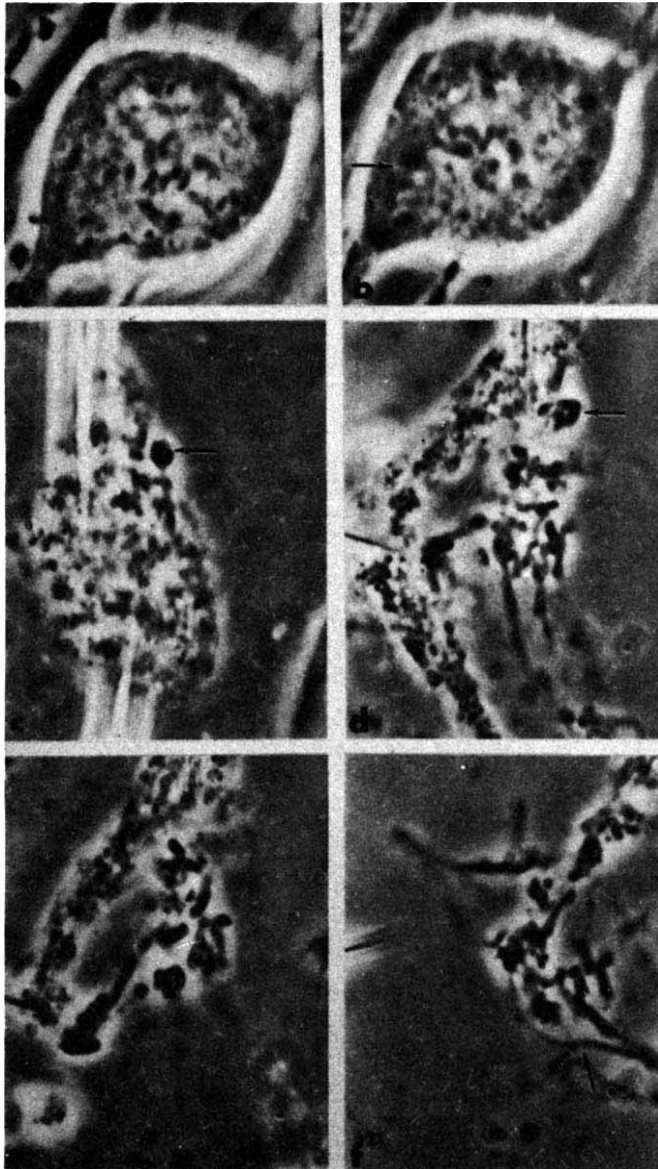


Fig. 1 Chromosome extraction from an HEL prometaphase cell shown before operating in *a*. Chromosomes were displaced to a position closer to the cell surface and the needle was removed. The displaced chromosomes are focused in *b* (arrow). If nothing further had been done, the cell would have completed mitosis. But the needle was reintroduced into the cell and chromosomes were extracted using two other needles to pin the cell against the coverslip *c*. In *c* a chromosome pair is in focus so that the ends of the four arms are visible. The chromosomes are more isolated in *d* and the chromosome pair shown in *c* has shifted (arrow) to the position shown in *d*. The other chromosomes are more clearly visible and there is evidence of adlinear connexions among them. Small grey spheres of detached hyaline cell matrix can be seen near the bottom of the figure. In *e* the chromosomes at the far left show a grey sheath enclosing them which is surrounded by nucleoplasm visible as a bright halo. In *f* the chromosomes are stretched and the connexions between them are still evident (arrow) ($\times 1,250$).

from the needle, it moves back to the main chromosome aggregate, as do the adjacent chromosomes. Linear measurements of these displacements are not made because of the continually changing shape of the cell as it progresses to metaphase.

By using fine, rigid needles, the cell deformation before penetration by a thicker needle is avoided. The use of needles which taper gradually reduces the likelihood that the medium bathing the cell will enter the cell. These observations are independent of the cell types studied, and it is possible to displace the chromosomes within a cell, allow the cell to divide

completely and then clone it. Two such clones have been obtained (Table 2).

If a chromosome is picked up on the tip of a microneedle and pulled through the surface membrane, this chromosome will remain outside but in intimate contact with the cell surface. The adjacent chromosomes are then visible as a bridge extending from the main group of chromosomes to the cell surface. Division may or may not continue.

If, on the other hand, a chromosome is extracted or moved a short distance from the cell surface, other chromosomes can be extracted merely by moving the first extracted chromosome further from the cell (Fig. 2). A chromosome trapped halfway out of the cell will move back into it if the tension is decreased.

In HeLa cells, "persistent" nucleoli can be removed without losing their intracellular geometry (Fig. 3) as chromosomes are extracted. In cells of malignant origin which are binucleate, it is also possible to remove the chromosomes of one nucleus without removing the chromosomes of the other (Fig. 3).

The greatest difficulty in the procedure of extracting chromosomes is to separate the chromosomes which have been extracted from those which remain inside the cell. Leaving extracted chromosomes at the cell surface severely disturbs the geometry of the cell. We observed four such cells and they all reverted to an interphase state. The extracted chromosomes were obscured and the cells died shortly afterwards.

In metaphase, the spindle region can be probed or chromosomes impaled with fine needles and division will continue in all three cell types (Table 1). But chromosomes cannot be extracted with needles as in prometaphase. To release the chromosomes from a cell in metaphase, the method which follows was devised.

In a metaphase HEL cell which has not been punctured, a silicone oil drop of diameter approximately equal to that of the cell is expelled from the tip of a pipette onto the cell surface between the axis of the metaphase plate and one of the poles

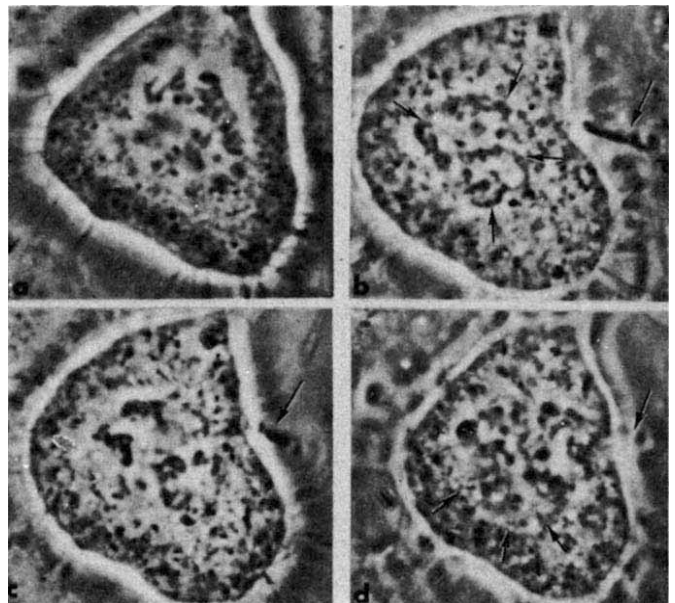


Fig. 2 The chromosomes of the ERK prometaphase cell shown in *a* are shown. A needle was then brought into the cell and chromosomes were pulled through the cell surface. In *b* two of three extracted pairs are visible (arrow) and are connected to the first pair (not shown) attached to the needle. To the left of these chromosome pairs in an almost straight line are the chromosome pairs within the cell but displaced toward the extracted chromosomes. The intracellular chromosomes show an S-shaped configuration (arrows). The extraction was continued, and in *c* another pair (arrow) has been removed by lowering the needle and moving it farther away from the cell. The chromosomes visible in *b* are now below the plane of focus. In *d* the chromosomes have been freed from the needle and are hanging from the cell (arrow) below the plane of focus. Chromosomes within the cell give the nuclear region a reticulated appearance because of their interconnexions. Note the connexions between the nuclear region and the granular cytoplasm (arrows).

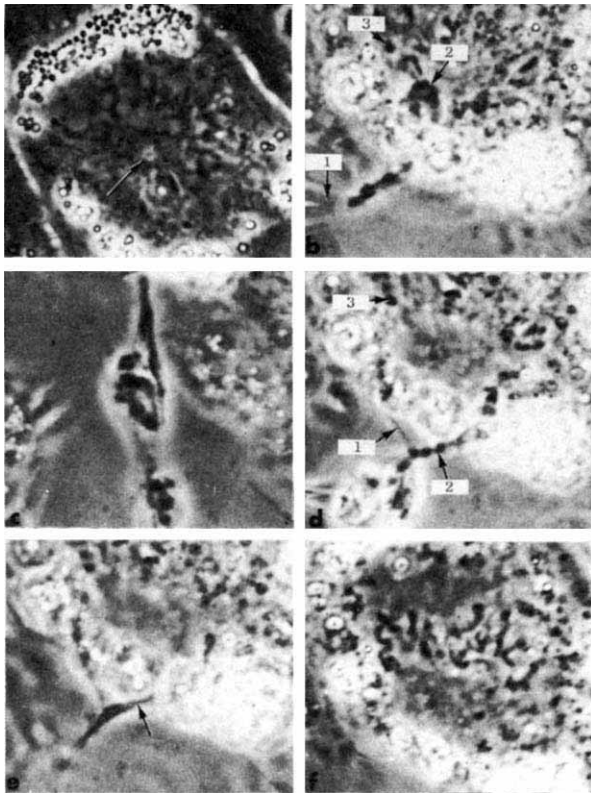


Fig. 3 A prometaphase HeLa cell is shown in *a* and the region corresponding to the Golgi region is evident from the light centre (arrow) around which the visible cytoplasmic structures appear to be radially arranged. A needle was brought into the cell after this photograph was taken and chromosome pairs were pulled through the cell surface. The tip of the needle (1) is shown in *b* with the extracted chromosomes to the right of it. A persistent nucleolus (2) is within the main chromosome group, and (3) is a chromosome at the periphery of the main group. In *c* the nucleolus shown in *b* (arrow 2) is outside the cell; the relative positions of the chromosomes within this structure have not changed (compare with *b*). Although chromosomes were extracted from only one small area through the cell surface, chromosomes began to emerge from another part of the cell surface. In *d* the Y-shaped configuration is shown (arrows 1 and 2). Arrow 3 corresponds to arrow 3 in *b*. In *d* the nuclear volume is reduced to the clear region previously occupied by the extracted chromosomes. In *e* the chromosome pair has connections between the proximal arms (nearer the cell) and a structure still within the cell (arrow). In *f* the central region of the cell is shown emptied of chromosomes adjacent to the region in which chromosomes are still present ($\times 937.5$).

(Fig. 4). The chromosomes move toward the base of the oil drop and are extruded. They may remain at the cell surface or be expelled completely. The entire chromosomal complement of the cell can be removed in this way and the chromosomal aggregate can be attached to the surface of the coverslip for further manipulation. Once the chromosomes are released, individual pairs can be dissected free of the main group and aspirated into a pipette for transplantation.

Smaller drops of silicone oil will not induce this reaction. Larger drops cause contraction of the cell which leaves the surface membrane free of the contracted cytoplasm except for minute linear connexions. An oil drop poised on the pipette and brought into contact with the cell surface does not elicit chromosome expulsion.

For HeLa and ERK cells in metaphase, this procedure is ineffective regardless of the size of the oil drop or its position on the cell surface, and so we injected an aqueous fluid at the visible demarcation between the granular cytoplasm and the clearer spindle region. The cell could then be torn open and the chromosomes released (Fig. 5).

Until the metaphase configuration of the living mitotic cell is expressed, polar chromosomes, seen in HeLa and ERK cells, cannot be delineated. In metaphase and after, however, chromosomes which do not take part in the mitotic movements of most chromosomes are visible as rounded, dark bodies embedded in hyaline material. They may appear in any plane of the polar region of the cell, and may be asymmetric in configuration or unequal in numbers at two or more poles. They may be probed without disrupting mitosis.

Chromosomes can be probed and the spindle region probed in HEL, ERK, and HeLa cells without disrupting mitosis (Table 1). Expelling oil drops against the equatorial or polar surface of the cell will not induce extrusion of chromosomes. In early anaphase, chromosomes of HEL cells seem to separate in a spherical configuration. In HeLa and ERK cells, the large number of chromosomes tends to obscure the sphericity. Injection of aqueous fluids at the granular interface between cytoplasm and spindle will free the chromosomes as two groups, one on either side of the elongate, achromatic structure. The rigidity of the spindle and the size of the unpaired chromosomes make them more difficult to dissect free than at metaphase.

Movement of chromosomes from one group to the other within the cell is prevented by the extreme rigidity of the achromatic spindle. If a chromosome is impaled and pulled across the spindle, cell deformation occurs and the cell is destroyed because it is torn in the process. Moreover, in heteroploid cells, HeLa and ERK chromosome bridges are under considerable tension as the distance between the two chromosome groups increases.

Chromosomes of all three cell types can be probed in telophase before the nuclear membranes are visibly reconstituted, and cytokinesis will continue. If the needles are used to probe the region where the division furrow will form, cytokinesis

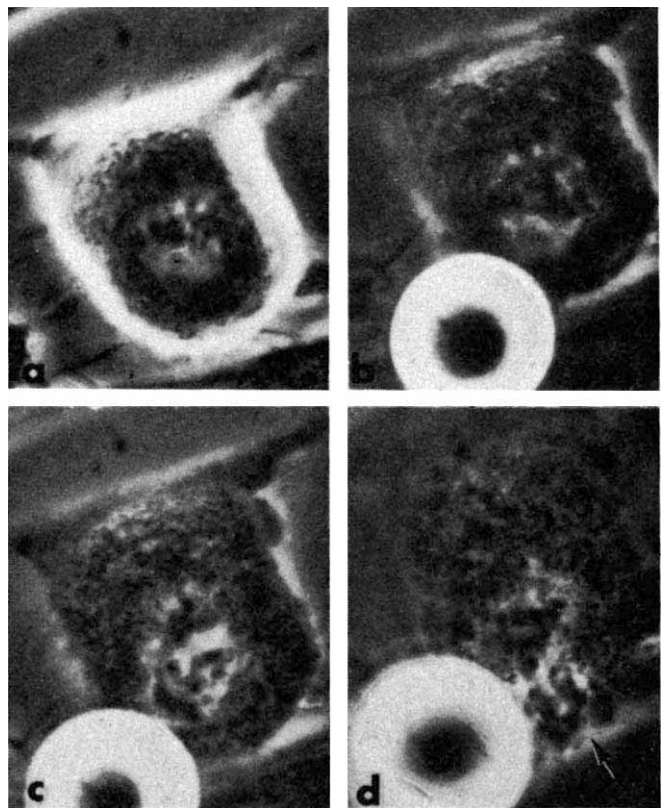


Fig. 4 Different stages during the release of chromosomes from the unoperated HEL metaphase cell shown in *a*. An oil drop was expelled onto the cell surface *b*, and the chromosomes are already displaced toward the oil. In *c* the chromosomal mass is almost at the site where the oil drop has attached to the surface of the cell; and in *d* the chromosomal aggregate has broken through the cell surface (arrow).

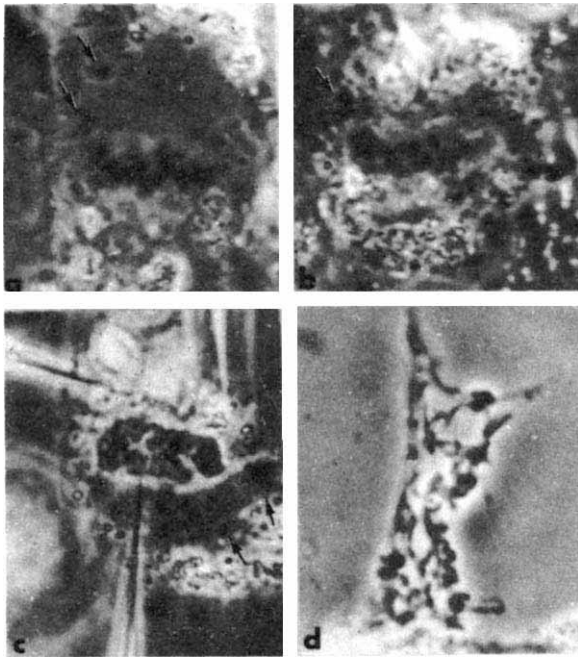


Fig. 5 The stages in releasing chromosomes from either HeLa or ERK cells in metaphase are shown in this figure. The HeLa cell shown in *a* has polar chromosomes (arrows) which remain in this relative position throughout the operation. Aqueous fluid was introduced, and in *b* the region containing the chromosomes is compressed; the arrow indicates the polar chromosome nearer the main mass in *a*. During the tearing of the cell, it has been rotated 180°, and the chromosomes (arrows) in *a* are indicated in *c* (arrows) maintaining their positions. In *d* the extracted chromosomal aggregate has been expanded using three needles (not shown) and the chromosomes are shown in a three-dimensional network lying free of the cell and held near the coverslip by the needles ($\times 1,125$).

seems to be accelerated and separation of the daughters continues.

Oil drops do not induce chromosome extrusion nor can chromosomes be extracted with microneedles. Injection of aqueous solutions at the boundary between nucleus and cytoplasm frees the chromosomes. Probing the nuclei during reconstitution is not lethal. The rosette arrangement of the chromosomes in HEL cells is clearly visible, although the pattern is obscured in HeLa and ERK cells, which may have chromosome bridges in telophase. These structures are under considerable tension and can be severed completely or partially with a microneedle. If a bridge involving one or two chromosomes is cut, the severed members rapidly retract to their respective poles. If more chromosomes are involved, cutting is more difficult and the tension on the remaining chromosomal bridge seems to be increased. Furthermore, chromosome bridges may persist and the cells alter their configuration to accommodate such bridges.

These studies show that (a) human somatic cells will survive exposure to light (compare with ref. 7) and a temperature which slows but does not stop mitosis; (b) mitosis is a phase of the cell cycle amenable to and not easily disrupted by the operations described; and (c) cell size and chromosome number do not impede basic, exploratory procedures (compare with ref. 8).

What features have been revealed by these studies? In general, human mitotic cells represent complex topological systems of constantly changing shape and surface area. As a result, linear measurements useful for study of insect eggs^{9,10} may not be as meaningful for the study of human somatic cells.

Chromosome extraction during prometaphase gives the impression that there are connexions among chromosome pairs other than those between spindle fibres and chromatids. Additional evidence comes from studies of non-randomly associated, satellited chromosomes or fragments¹³⁻¹⁵, chemical treatment of chromosomes^{16,17}, and of persistent nucleoli^{11,12} or of interphase chromosomes by ultracentrifugation¹⁸ and electron microscopy^{15,19}.

Chromosome release from HEL cells in metaphase after the application of oil to the cell surface is not easily explained. Several interpretations may involve membrane potentials, surface charge, denaturation of proteins or surface tension, in accord with the differences noted between the response of HEL compared with HeLa or ERK cells and studies of cell surfaces²¹⁻²⁴. The differences in the surface reactivity in a cell as mitosis progresses may prove to be more significant than the difference between the fibroblastic architecture of HEL cells and the epithelioid architecture of HeLa or ERK cells. Although there is no obvious simple explanation, the method of releasing chromosomes from metaphase HEL cells is easily reproducible and chromosomes free of granular cytoplasm²⁴ can be acquired for more detailed examination.

Short term experiments on human mitotic cells²⁵⁻²⁸ reveal subcellular organization, and the study of several living cells during mitosis reveals architectural changes⁵ within living cells that are not evident in fixed material.

The tensile strength of chromosome bridges is appreciable and may have significant effects on the post-mitotic configuration or nuclear morphology of the daughter cells.

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LETTERS TO NATURE

PHYSICAL SCIENCES

The World Rift System and Plate Tectonics or 1971 and All That

"I'll put a girdle round about the earth in forty minutes"
(Puck)¹

They put a girdle round the Earth
And named it the Worldwide Rift;
It helps explain the ocean floor
And Continental Drift.

Vine and Matthews sailed away
Exploring the ocean bed;
It took much longer getting them back;
They said it was seafloor spread.

It appears that the oceans were mostly formed
By Cenozoic streams
Of mantle flooding up the cracks
And gumming up the seams.

Our Earth can scarce make up its mind;
It flips its magnetic poles
And the magnetized basalts so produced
Give temporal controls.

When all believed the Earth was flat
It was but a single plate,
But even then the edges were
Hot subjects for debate.

They can't curl down; they must curl up
To form a kind of dish
To stop the oceans spilling out
And losing all the fish.

We now all know the Earth is round
And moves about the Sun²,
So what the ocean spreaders said
Could not be simply done.

Unless the Earth itself enlarged
And kept on getting bigger,
But gravity opposes this:
It is a constant figure.

And so the plate has been revived
In present day tectonics,
Though sial and sima still remain
As crustal term mnemonics.

Both kinds of crust now constitute
The grander types of plates
And as they move upon the Earth
They suffer subtle fates.

The edges which are growing still
Are hid beneath the oceans,
While those around the island arcs
Show self-consuming motions.

Yet others seem to hit or slide
Performing curious functions,
And where they can't make up their minds
You there have triple junctions.

On continents the crustal plates
Are edged by earthquake foci,
And little plates proliferate
By joining up the loci.

As alchemists once sought the stone
For magic transmutations,
The motions of the plates are shown
By seismic computations.

Geologists naively thought
That rifts were due to faulting,
By subsidence of crustal strips
Along a pre-rift vaulting.

Their evidence was solely based
On visual observations
Of structure and stratigraphy
And such out-moded notions.

But others now hold better views
And think that each "mañana"
Brings Africa a step more close
To the fate of old Gondwana.

McKenzie sees his moving plates
Wedging the rift asunder,
And one day ships will sail the rift
To maritime Uganda.

Girdler and Khan from the gravity highs
That they have in the Gregory rift
See the mantle rearing its ugly head
And East Africa going adrift.

But the OAU no doubt will vote
At a suitable early date
To stop that drift and plug the rift
Before it is too late.

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Received May 7, 1971.

¹ Shakespeare, W., *A Midsummer Night's Dream* (1599).

² Copernicus, N., *On the Revolution of the Celestial Orbs* (1543).

Other, more familiar, references will be found in recent issues of *Nature*.

Addendum: Headline in *The Times*, June 18, 1971, page 6:
"Emperor tries to close African rift."

Rapid Variations in the High Energy X-ray Flux from Cyg X-1

THE intensity of X-rays in the energy range 22.5–154 keV from the X-ray source Cyg X-1 was monitored for about 3 h on the night of April 5, 1971, using a balloon-borne telescope. There were pronounced variations in the intensity and presumably energy spectrum of the source in time periods of the order of a few minutes.

The X-ray telescope was similar to that flown by us¹ before, and comprised an NaI(Tl) crystal of effective area 97 cm² provided with active and passive collimators and having an FWHM of 18.6°. The telescope was mounted on an orienting device with its axis at an angle of 17° with respect to the zenith and was programmed to point alternately for 10 min due north ($\phi = 0^\circ$, B mode) and then for 5 min 42° east of north ($\phi = 42^\circ$, A mode) during the whole flight. The stabilizer of the detector and the associated electronics was checked by means of a ²⁴¹Am source which came into the field of view of the telescope for about 30 s once every 15 min and provided in-flight calibration at energies 16.5 and 59.8 keV.

The balloon was launched from Hyderabad (17.6° N, 78.5° E) at 2119 UT and reached the ceiling altitude of 5.2 g cm⁻² at 2330 UT, when it floated at constant altitude until 0315 UT on April 6, 1971. The altitude was measured accurately to ± 0.05 mm Hg using a Wallace Tiernan photobaro-graph. The pulse amplitudes from the X-ray detector were sorted into fifteen channels and recorded on-board and also telemetered together with aspect information obtained from crossed magnetic sensors. The time of occurrence of each event was registered to an accuracy of 1 ms using a crystal controlled digital clock with an accuracy of 1 in 10⁶.

The data are presented in Fig. 1. The meridian transit of Cyg X-1 at Hyderabad was at 0200 UT and the zenith angle of the source at meridian transit was 17.9°. In the earlier part of the observations, Cyg X-1 was seen more efficiently in the

A mode and in the later part in the B mode. The calculated exposure efficiency of the telescope for recording X-rays from Cyg X-1 in the two modes is also shown in Fig. 1, together with corresponding estimates for Cyg X-2 and Cyg X-3, which are considerably weaker sources at balloon altitudes.

The background counting rate of the telescope has been obtained as follows. During the periods marked I, II and III in Fig. 1, the efficiency (η) for Cyg X-1 in the B mode was much less than 10%, and Cyg X-2 and Cyg X-3 were not in the field of view. In the intervals marked IV, V, VI and VII, the efficiency for Cyg X-1 and Cyg X-3 was less than 10% in the A mode, but in later intervals the efficiency for Cyg X-2 is of the order of 30–40%. The counting rates in these intervals are, however, comparable with those in I, II and III, from which it is concluded that the contribution of Cyg X-2 was quite negligible, so that the average of the counting rates of I to VII has been used as the background counting rate. The background counting rate for the energy interval 22.5–99 keV is 873 ± 5 per minute and for the energy interval 99–154 keV the counting rate is 765 ± 5 per minute. The excess counting rates over the background for all the intervals for which the efficiency for Cyg X-1 was higher than 25% and time of observation at least 2 min have been listed in Table 1 along with other relevant details. The actual pointing times in the two modes A and B were 5 min and 10 min respectively, but a certain fraction of the time was invariably lost either because of the appearance of the in-flight calibration source which was synchronized with the A mode or due to time lost in the change over of magnetic tapes while recording. As accurate time markers were always available, it was possible to determine the exact exposure on the source for each interval.

Many investigators^{2–4} have claimed that the intensity of Cyg X-3 is less than a tenth of Cyg X-1, but the observations of Rochia *et al.*⁵ and Peterson⁶ show that on occasions the intensity could be as high as a third or a fifth of Cyg X-1. It is therefore necessary to take account of the possibility of a

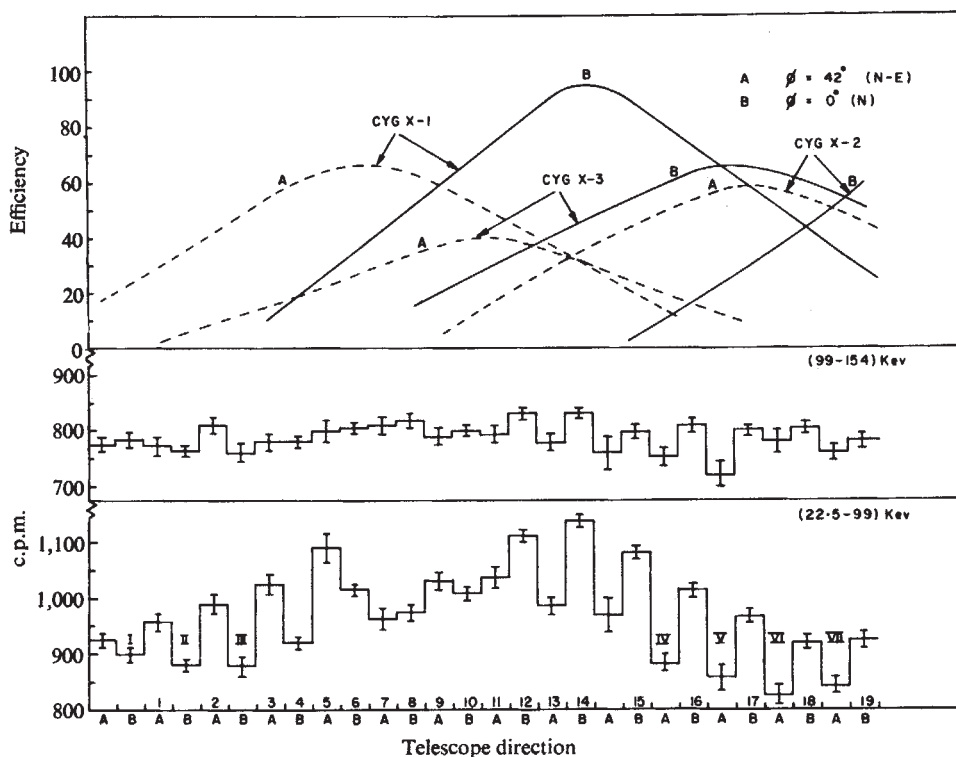


Fig. 1 The telescope counting rate in the two energy bands 22.5–99 keV and 99–154 keV as a function of time between 2346 and 0315 UT. The direction of the telescope was alternated between due north (B mode) and 42° east of north (A mode). The efficiency of the telescope for recording X-rays from Cyg X-1, Cyg X-2 and Cyg X-3 shown is at the top for the two modes as a function of time.

Table 1 Excess Counting Rates

Serial No.	Telescope mode	Time UT April 6, 1971	Time of observation (min)	Efficiency of detection		Excess per min		Excess per min normalized to Cyg X-1 efficiency	
				Cyg X-1	Cyg X-3	22.5-99 keV	99-154 keV	22.5-99 keV	99-154 keV
1	A	0002-0007 *	4	30	3	80 ± 16	9 ± 15	268 ± 53	28 ± 50
2	A	0017-0022 *	4	42	8	116 ± 16	46 ± 15	276 ± 37	109 ± 36
3	A	0032-0036 *	3	55	15	150 ± 19	17 ± 17	273 ± 34	31 ± 31
4	B	0036-0046	9	20	—	146 ± 11	16 ± 10	230 ± 55	80 ± 50
5	A	0046-0051 *	2	64	21	162 ± 24	34 ± 20	253 ± 37	53 ± 31
6	B	0051-0102	9	34	7	132 ± 12	40 ± 20	388 ± 35	118 ± 29
7	A	0102-0107 *	3	65	30	92 ± 18	43 ± 17	141 ± 28	66 ± 26
8	B	0107-0117	6	50	15	100 ± 14	54 ± 12	200 ± 28	108 ± 23
9	A	0117-0122 *	4	59	38	158 ± 16	23 ± 15	268 ± 27	39 ± 25
10	B	0122-0132	10	65	26	134 ± 11	34 ± 10	206 ± 17	52 ± 15
11	A	0132-0137 *	3	49	37	163 ± 19	28 ± 17	333 ± 37	57 ± 35
12	B	0137-0147	10	83	35	237 ± 11	65 ± 10	286 ± 13	79 ± 12
13	A	0147-0152 *	4	37	34	113 ± 16	12 ± 15	306 ± 42	32 ± 40
14	B	0152-0202 †	10	94	47	262 ± 11	65 ± 10	279 ± 11	69 ± 11
15	B	0207-0217	7	84	57	206 ± 13	31 ± 12	246 ± 16	37 ± 14
16	B	0222-0233	6	74	63	240 ± 14	42 ± 13	324 ± 19	57 ± 18
17	B	0237-0247	8	58	64	94 ± 12	34 ± 11	162 ± 20	59 ± 17
18	B	0252-0302	7	42	59	47 ± 13	38 ± 12	112 ± 30	91 ± 29
19	B	0307-0315 *	5	28	50	51 ± 14	16 ± 14	182 ± 50	57 ± 50

The table gives excess counting rates for intervals for which the exposure efficiency for Cyg X-1 was higher than 20% and time of observation more than 2 min. Of the 8 time intervals missing from the table between 0002 and 0315 UT, 7 have been used for the background determination as explained in the text.

* Calibration source in the field of view. † Data for time interval 0202-0207 not presented as observation time was less than 1 min.

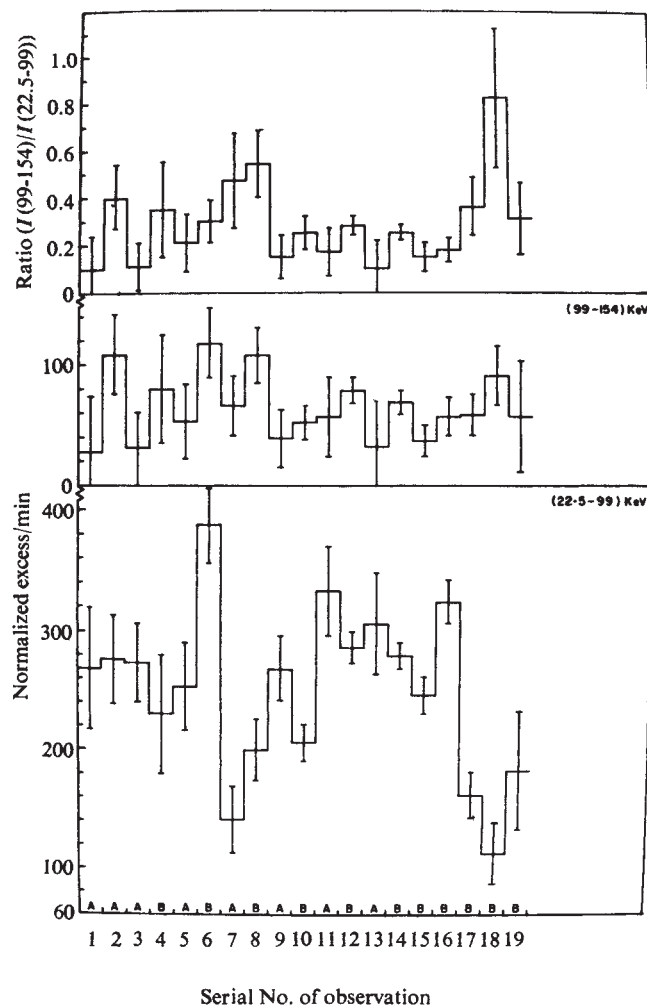


Fig. 2 Normalized excess counting rate (normalized to 100% efficiency of Cyg X-1) as a function of the serial number of observation for the two energy bands 22.5-99 keV and 99-154 keV. The UT corresponding to the different observations are given in Table 1. The histogram at the top gives the ratio of the counting rates in the two energy bands for the different observations, which is a crude measure of the spectral index.

relatively high intensity for Cyg X-3 (say, one third) while discussing the time variations of Cyg X-1 observed in the present investigation. We shall consider all those intervals for which R_η , the ratio of the exposure efficiency of Cyg X-3 to Cyg X-1 is less than 0.5 so that the relative contribution to the excess counting rate by Cyg X-3 is < 17% of Cyg X-1. Table 1 shows that all the observations except 9 satisfy this criterion. For these intervals as well as for all the others the contribution of Cyg X-2 can be completely ignored, for reasons already stated. In Fig. 2 we have plotted the normalized excess counts (normalized to 100% efficiency of Cyg X-1 ignoring Cyg X-3) as a function of the serial number of the observation. The universal times corresponding to the various observations are given in Table 1. It is seen that in the range 1-12 there are wide variations in the normalized excess counts in the lower energy band. The variations are not so pronounced in the higher energy band. The excess count in the observation 6 in the lower energy band is 388 ± 32 counts per minute while in the neighbouring observation 7 which is the very next interval in time also the excess drops to 141 ± 28 counts per minute. The mean excess in the range 1-12 (excluding 9) is 260 counts per minute for this energy band. In the observations 13, 14, 15 and 16 ($R_\eta > 0.5$) there is no significant variation in the excess counting rate. Observations 17, 18 and 19, however, show considerably lower values of excess counts (the mean value for these intervals being 152 ± 14). In these cases, as can be seen from Table 1, $R_\eta > 1$ and consequently the normalized excess given in Fig. 2 would be an upper limit as far as the intensity of Cyg X-1 is concerned. Therefore, during this period it can be concluded that the intensity of Cyg X-1 has decreased from the mean value corresponding to (1-12) by a factor of ~ 2 . The observations 16 and 17 are consecutive in time and therefore the fall in intensity has taken place within a few minutes. If, on the other hand, we assume that the intensity of Cyg X-1 is constant and attribute the variations to Cyg X-3, then the extent of variations in the intensity of this source will have to be more than a factor of 20 which seems to be extremely unlikely.

In Fig. 2 we have also plotted the ratio of the counting rates $I(99-154)/I(22.5-99)$ as a function of the serial number of observation. The variation of this ratio is a crude measure of the variation of the spectral index. It is seen that the ratio does show some variations also indicating probable changes in the spectrum (detailed spectral variations will be published later).

Variations in the intensity and spectrum of Cyg X-1 over periods of 10^3 s in the energy range 2.5-9 keV have been

reported recently by Oda *et al.*⁷ based on their observations with the X-ray telescope on the UHURU satellite. The present results show that such rapid variations are seen at higher energies also. It would be interesting to see whether the variations in the different bands are time correlated.

Cyg X-1 has been extensively studied at balloon altitudes^{2-6,9,10}. The possibility of long term variations, over periods of several weeks, in the intensity of Cyg X-1 was first pointed out by Overbeck and Tananbaum² and has been further supported by Bingham and Clark⁴. Haymes *et al.*^{9,10}, however, found no substantial difference in the intensity and spectrum of Cyg X-1 between two flights—the first made on August 29–30, 1967, and the second on June 4, 1969. From a compilation of all existing data, Dolan⁸ has attempted to discern a periodicity of several days to examine the hypothesis of an eclipsing binary system as a possible cause of the variability. In the light of the results of Oda *et al.* and of the present experiment, the long term variations might be the result of superposition of short term variations of a few minutes and much of the anomaly in the long term variations may be because of this reason. In some of the earlier balloon experiments referred to above, long continuous exposures have been obtained on this source. The data, however, seem not to have been analysed specifically to look for variations in intervals of the order of minutes. A re-analysis of the data would be highly rewarding since that would establish whether the variations of the type observed in the present experiment are of recent origin or have been there all the time.

Oda *et al.*⁷ have reported the evidence for a pulsating component with a period of ~ 73 ms (or multiples of 73 ms up to 292 ms) in the X-ray emission from Cyg X-1. The analysis of Holt *et al.* (Goddard Space Flight Center preprint No. X-661-71-127) of their rocket data on Cyg X-1 has confirmed the presence of a periodic component in the X-ray emission from Cyg X-1, but suggests a double periodicity one of 290 ms and another of 1.1 s modulating each other. Our data are being put through a fast folding analysis to examine whether any periodic components are present at higher energies also.

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Interstellar Dust Clouds

THE failure, thus far, to detect gaseous emission lines from circumstellar dust clouds¹⁻³ and from Bok globules² raises the possibility that such clouds consist solely of dust grains. The possibility has previously been largely discounted on the grounds that relative diffusion of the grains and gas is slow and inefficient requiring time scales which are too long and resulting in only partial separation of the components^{4,5}. This communication points out that a process of forced diffusion will occur in clouds of grains and gas in which stars have formed and will result in a complete separation of the components in a relatively short time.

Atoms and molecules striking the grains in a well mixed cloud of gas and grains will momentarily stick on the surface and some will be adsorbed to remain there semipermanently. The binding energy of the adsorbed atoms and molecules will be in the range of 20 kcalories mol⁻¹, appropriate to hydrogen atoms on a graphite surface, through 2 kcalories mol⁻¹ for hydrogenic molecules⁶, to 200 calories mole⁻¹ for hydrogen on a hydrogen surface⁷.

Radiation from a star formed in the cloud will photo desorb gas from the grain surfaces. The time between arrival of a photon on the grain surface and departure of the adsorbate will be comparable with, or less than, the reciprocal frequency of vibration of the adsorbate⁶, 10^{-11} – 10^{-13} s. The grains that are in thermal kinetic equilibrium with the gas will have speeds of translation and of surface rotation $V \sim \sqrt{(3kT/m)}$ where T is the gas kinetic temperature and m is the mass of a grain. T will be less than the maximum surface temperature of stars, $\sim 4 \times 10^4$ K, and the mass of an interstellar grain of dimensions $\approx 10^{-5}$ cm is $\approx 10^{-15}$ g. Consequently $V \leq 130$ cm s⁻¹ and the period of rotation of a grain $t_{rot} \geq 6 \times 10^{-7}$ s, very much larger than the photo desorption time. Consequently there will be insignificant rotation of a grain in the time between arrival of a photon on the grain surface and the resulting photo desorption. Molecules and atoms will therefore always be photo desorbed from the grain side facing the source of the radiation. The result will be a rocket effect with gas streaming inwards towards the star and grains recoiling outwards. Inward moving gas will collide with the outward moving grains but will be subject to the same process and will not transfer any net momentum to the grains. This will also be true of thermal desorption since it will not have the directional property of the photo desorption. The forced diffusion driven by the stellar radiation inevitably continues until there is complete separation of the gas and grains. In the case of a roughly spherical cloud with a central star this will ultimately result in the dust cloud surrounding the gas cloud.

A binding energy of ≤ 2 kcalories mol⁻¹ is equivalent to ≤ 0.1 eV per molecule, corresponding to the energy of a photon emitted from a surface at 10^3 K; when the temperature of the central star reaches 10^4 K, most of the energy will go into kinetic energy if each photon detaches only one atom or molecule, the initial velocity of an H₂ molecule towards the star being $V_1 \approx 10$ km s⁻¹. The initial velocity will be less before this temperature is reached. The final layer of atoms, chemisorbed, will have binding energies ≈ 1 eV and will only be desorbed when the temperature of the star exceeds $\sim 10^4$ K.

If n_c is the number of gas atoms or molecules striking a grain in unit time and n_d the number photo desorbed, the net inward

speed of flow of the gas is $\frac{1}{\pi} n_d V_1 / n_c$ km s⁻¹. If n_p is the rate of

arrival of photons on the grain then $n_d = \alpha f n_p$ where α is the photo desorption coefficient and f is the fractional coverage of the surface by adsorbate. Here $\alpha = n_s \int \sigma(\lambda) r(\lambda) d\lambda$ where n_s is the number of atoms and molecules per unit area in a saturated adsorption layer (for which $f=1$), $\sigma(\lambda)$ is the photo desorption cross section, and $r(\lambda)$ is the normalized distribution of intensity of the incident radiation. n_p will depend on the

distance from the star. For a circumstellar dust cloud a grain of cross section 10^{-10} cm² at a distance 10^{18} cm from an OB star emitting 10^{50} photons s⁻¹ of average energy 3 eV, $n_p \sim 10^3$ s⁻¹. For a gas cloud of density 10^4 cm⁻³ and $T_k \leq 10^4$ K, $n_e \lesssim 10$ s⁻¹. Unless $\alpha < 10^{-2}$, atoms and molecules will be photo desorbed from the half of the grain facing the star as quickly as they are adsorbed.

The sticking coefficient will generally be high, especially when $T_k \lesssim 100$ K, and most incident gas will be adsorbed; consequently $n_d/n_e \sim \frac{1}{2}$ and the inward flow of gas relative to the grains will be ~ 1 km s⁻¹. The time scale for separation of the gas and grains in a circumstellar cloud 1 pc in radius will then be $\sim 10^6$ yr.

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Plate Tectonic Models for Orogeny at Continental Margins

In the theory of plate tectonics convergent plate junctures are the loci of orogeny¹, marked superficially by arc-trench systems², and the margin of one plate is consumed in a trench subduction zone. The overriding or consuming plate margin is characterized by a magmatic belt supporting both arc volcanism and batholithic intrusion at crustal levels. For rock assemblages formed within active arc-trench systems, the deformation and metamorphism of orogeny is a normal consequence or corollary of origin. Orogenic belts, however, also contain rock assemblages that were not formed within arc-trench systems, but only incorporated within them by subsequent events³. Prominent among these are the continental terraces—miogeoclinal assemblages and their associated continental rises formed along stable continental margins at continent-ocean interfaces within plate interiors. Several models, each consistent with plate tectonic logic, can be invoked to explain the eventual orogenic deformation of miogeoclinal continental margins.

There are two principal alternatives. On the one hand, the inactive continental margin on which the miogeoclinal assemblage accumulates may be activated as the consuming or overriding plate margin of a newly initiated arc-trench system. This alternative, which I shall call the activation model, implies that the ensuing orogeny results from some new pattern of plate boundaries that did not exist before the orogeny. Others have discussed the likelihood that newly required zones of plate consumption might well be localized along continent-ocean interfaces⁴, where contrasts in the architecture of the lithosphere are perhaps greatest. Depending on locally variable relations between the geometry of subduction and arc magmatism, compared with the geographic configuration of the pre-existing continental margin, the miogeoclinal terrace and the adjoining continental rise may be deformed in any of three ways according to the activation model. Deformation could be caused (1) by subduction and slicing in a trench regime, (2) by intrusion and metamorphism in the roots of a magmatic

arc, or (3) by folding and thrusting at the back flank of the arc structure along the edge of a foreland basin^{1,2}.

Alternatively the quiet continental margin with its miogeoclinal wedge of strata may ride against an arc-trench system at a convergent plate juncture through consumption of the intervening oceanic crust forming the basin offshore from the miogeoclinal wedge. In this case, which I will call the collision model, the miogeoclinal assemblage is partially subducted until the buoyancy of the thick continental crust chokes the subduction and presumably forces readjustment of plate boundaries. This alternative implies that the orogeny results simply from the continuation of a given pattern of plate motions to an inevitable ending.

The attachment of the Indian sub-continent to Eurasia is an appropriate example of continental collision. From the times of its final separation from Gondwanaland in the late Mesozoic, palaeomagnetic measurements show that India then moved on a generally northward course and finally linked up with Eurasia in the late Caenozoic⁵. The join between India and Eurasia is marked by the ophiolitic melange belt along the Indus Suture Zone, and the collision is reflected by telescoping of Phanerozoic sedimentary strata and their subjacent continental basement in the Himalayas to the south⁶. The once intervening ocean was presumably subducted beneath Tibet from Cretaceous to Miocene time, following which the continental edge of India was wedged beneath the Tibetan Plateau, which is probably as high as it is simply because of the doubling overlap of slabs of continental crust. As no magmatic arc is active in the region now, the attempted subduction of India has presumably choked the subduction zone along the suture, and a new convergent juncture may now be in process of formation along an anomalous seismic zone stretching south-east from Ceylon⁷. The root of the fossil magmatic arc along the southern margin of the Tibetan block is represented by granitic batholiths of Mesozoic and Caenozoic age⁸ (about 210 to 215, 185, 130 to 135, 90, 50, 30 and 15 m.y.) in the Hindu Kush and Karakoram ranges just north of the Indus Suture Zone. An older example of continental collision is the Urals, where progressive accretion of oceanic materials to at least one of the approaching continental margins preceded the collision of Europe and Siberia in the late Palaeozoic⁹.

If orogeny is a collision between a quiet continental margin and an intra-oceanic island-arc system, termination of subduction along the near side of the arc may be succeeded by a simple flipping of the subduction zone to a position on the far side of the arc. In this case, a newly accreted arc simply becomes the newly active edge of the continent, although the inclined seismic zone associated with post-collision subduction dips in an opposite sense to the one associated with pre-collision subduction. Consumption of nearby oceanic crust continues in spite of the collision. The north coast of late Caenozoic New Guinea has been suggested as an example of the working out of the model on the edge of the Australian continent¹⁰.

The multiple thrusting and probable metamorphism that would accompany the attempted insertion of a miogeoclinal wedge into a subduction zone according to any collision model invite confusion with thrusting in either the trench or arc-rear positions inherent in the activation model. Either model can also lead to the construction of an "exogeosynclinal" clastic wedge across the deformed miogeoclinal prism and its adjacent craton. The sources of the sediment would be the marginal arc or associated highlands behind it in the activation model, and the collided arc or associated highlands in front of it in the collision model. Successor basins built on top of "geosynclinal" strata could also follow the culmination of either model. Consequently neither of the two models or their variants should be accepted as an explanation in a particular case without applying systematic logical tests for alternatives.

There are arguments that can be used to sort out pertinent orogenic events in time and space. First, the position of ophiolitic shreds, trench melanges or metamorphic rocks of blue schist facies indicating the position of fossil subduction

zones (where oceanic crust was consumed) will form a suture belt between miogeocline and arc-trench system in the collision model but will lie on the far side of the marginal arc in the activation model. Second, the potassicity of arc lavas and plutons increases in the direction away from the trench, as the depth to the inclined seismic zone increases, and can be used to establish the polarity of fossil arcs, which must face away from the miogeocline in the activation model but towards the miogeocline in the collision model. Third, the nature and facies of the rocks in the metamorphosed roots of fossil arcs may reveal whether the arcs were built through and on miogeoclinal wedges, or sutured against the miogeoclinal wedges during collision. Fourth, sedimentological data defining the source rocks of exogeosynclinal clastic wedges spread across a miogeoclinal sequence from a growing orogen may indicate whether the sediment was derived from partly indigenous material—as the activation model requires—or from partly foreign material, as the collision model permits. Fifth, orogenic events affecting the miogeoclinal margin will presumably have the same approximate duration as the life of the marginal arc in the activation model, but will be briefer and limited to the closing stages of arc activity in the collision model. Other tests may be devised in specific cases according to local constraints on timing and relative position.

There is a real possibility of confusion between the two models. Both east and west coasts of North America have been interpreted recently in both ways by different observers. The Piedmont of the southern Appalachians has been described provisionally as the late Palaeozoic active margin of North America¹¹, and also tentatively as a sutured piece of an African active margin detached from that continent by Mesozoic rifting following Palaeozoic collision with North America (H. L. James in his presidential address to the Society of Economic Geologists, November 1970). In the western United States, pre-Tertiary orogenic events have been related to the evolution of a complex marginal arc system¹² and also to a complex sequence of collisions¹³. In both these examples the divergent views expressed may ultimately be reconciled in terms of complex models, such as active continental margins face to face and flipping subduction zones bounding accreted arcs, for which neither simple activation nor simple collision is an adequate description. Marginal arcs that detach from continental edges to become fringing arcs are another factor.

No assumptions should be made without specific reasons or the plate tectonic theory of orogeny may lead to more confusion rather than new insight. Different models for different orogenic belts are inherent in the plate tectonic explanation of orogeny¹⁴.

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Periodic Solar Flare X-ray Emission

GLENCROSS¹ has reported the observation of an apparent 6 min periodicity during X-ray flares observed in the 0.1 to 0.3 nm wavelength band. The detector was a soft X-ray sensor on the satellite OSO-IV launch in 1967. The observed flare profiles were examined in more than thirty X-ray events coincident with visible flares, and a histogram was constructed showing the separation of peaks. The results showed such strong evidence for peak separations being multiples of 6 min that we began a search for similar peak spacing in X-ray data for a similar wavelength interval derived from scintillation detectors aboard the satellites Vela V (launched on May 23, 1969) and Vela VI (launched on April 8, 1970). The results of our search have not confirmed the 6 min periodicity.

The complement of solar X-ray detectors² carried by the Vela V and VI satellites includes a scintillator-photomultiplier whose output is fed to a five level, integral, pulse height analyser. The difference in counting rate between the 4.3 and 22.2 keV analyser levels gives a wavelength band of 0.06 to 0.29 nm approximating the OSO-IV data. For this study, real-time data (about 20% coverage, 1 min time resolution) were used. Vela V data for approximately 8 months beginning in May 1969, and Vela VI data for July 1970, were examined for X-ray events having multiple peaks. The selected events were those for which a single H α flare was listed³ and for which there were no comments such as "several eruptive centres", "very extensive active region", or "two or more brilliant points". Such comments indicate the possibility of more than one X-ray emitting region; we believe the theory proposed by Glencross to explain a periodicity can be valid only for single active regions. Thirty-eight events were found which seemed to be multiple X-ray events from a single source region, and the spacing between peaks was measured. The points at which the rate of flux increase became insignificant were used to determine the separation. In Fig. 1, which shows the distribution of time separations obtained, no marked periodicity is evident, and the only indication of concentration at a specific separation is at 3 min which is near the limit for resolution of two peaks in the Vela data. Thus, we have found no experimental support for the reported 6 min periodicity. Furthermore, if such a periodicity is found, one must be careful about identifying it with the chromospheric oscillation, since the velocity amplitude of the 5 min oscillation is observed to be greatly reduced in active regions⁴ and the oscillation may be altogether absent in regions of extremely large magnetic field.

In attempting to understand the presence of the marked periodicity which appears in the OSO data, we have investigated the H α flare list method we believe was used by Glencross to select data which represent X-ray emission from single, small active regions. We first noted that many times in our own use of flare lists, we discarded double X-ray events because the comment "two or more brilliant points" was made and

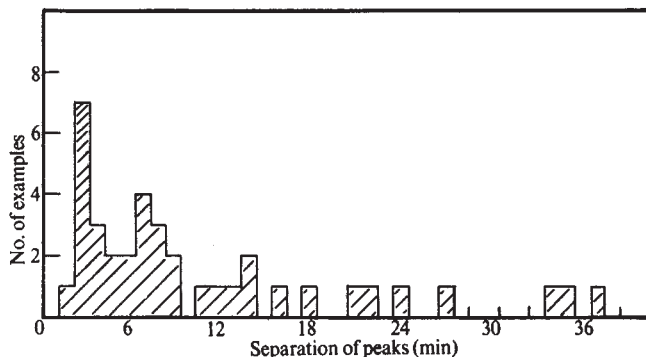


Fig. 1 Histogram of time separation between peaks of X-ray intensity for thirty-eight events.

the comment was made by only one, or a few, of a group of stations reporting. Thus, it must be difficult for observers to see both of two regions of brightness close together in space and time. Next we examined a flare patrol film from the Lockheed Solar Observatory, Los Angeles, which covers a double X-ray event with first peak at 1955 UT on June 6, 1969. We had not included this event in our tabulation because the second peak at 2002 UT corresponded to an unconfirmed flare³ observed by the Sacramento Peak Observatory, Sunspot, New Mexico. When we examined the Lockheed film, both the H α flare corresponding to the 1955 UT event and the separate H α flare corresponding to the 2002 UT peak event were readily visible to us at the locations shown in ref. 3. This experience indicates to us that it is possible for observers to overlook one H α flare when another region is also active and therefore that the absence of the listing of a second separate flare does not guarantee the presence of only a single region of activity.

Flare lists are apparently not adequate to insure selection of single flare events, and a significant X-ray enhancement may not be accompanied by observable H α emission. For these reasons we feel that Glencross may have included some multiple H α events in his histogram. It is not evident, however, that this could induce a 6 min periodicity, and the periodic peaks in the OSO-IV distribution remain to be explained.

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Anisotropy of Synthetic Metals

SYNTHETIC metals can be derived from graphite by intercalation of either electron donor or electron acceptor molecules between the carbon hexagon networks. In graphite, these networks are in effect macromolecules with aromatic character. Planar sheets of carbon hexagon networks lie parallel to the crystallographic *a*-axis of graphite, which has hexagonal symmetry.

Physical properties in directions parallel to the networks are thus "macro-aromatic" properties. Perpendicular to these sheets, in the direction of the crystallographic *c*-axis of graphite, physical properties involve much weaker physical interaction between the layers; probably only van der Waals dispersion forces hold neighbouring sheets together in graphite crystals. It is comparatively easy to account for the high *n* or *p*-electrical conductivities observed in the direction of the *a*-axis (as a consequence of charge transfer to or from the graphite electron bands¹) but effects of such charge transfer on electrical properties in the direction of the *c*-axis of the crystals cannot be predicted as readily. Even in the parent graphite, the anisotropy ratio of electrical conductivity σ_a/σ_c is about 4×10^3 for well aligned stress recrystallized material². It is possible that the relatively poor conduction of electricity in the *c*-axis direction in pure graphite involves a different kind of mobility, akin to electron hopping in crystals between molecules of poor conductors. In the synthetic metals the charged carbon hexagon networks are separated by positively charged layers of potassium atoms as in C₈K (ref. 3) or by negatively charged layers whose molecular composition is given by formulae such as C₂₄⁺HSO₄⁻·2H₂SO₄, C₂₄⁺NO₃⁻·3HNO₃, C₈Br, C₈ICl and so on. As far as conduction mechanisms in the *c*-axis direction are concerned, it is significant that considerable crystal swelling accompanies this intercalation; the repeat distance between successive sheets of carbon atoms increases from 3.35 Å in the parent graphite to a much larger separation, which is about 8 Å for graphite bisulphate⁴.

Hindrances to charge wandering across the layers in the *c*-axis direction of such crystal compounds must depend extensively on the electron affinity and the local repulsion potentials of the intercalate molecules. In view of the versatile crystal chemistry of these compounds, some interesting situations can be envisaged which involve such *c*-axis conduction, but until recently no reliable experimental data have been available. Earlier estimates⁵ were based on intercalates made from thick pyrolytic graphite "as deposited" by cracking methane gas. But in pyrolytic graphite, the distribution of crystallite *c*-axes with respect to the nominal *c*-axis direction is still far from exact parallelism. Because of this relatively poor distribution of orientations after intercalation of an electron donor or electron acceptor molecule the great increase in *a*-axis conduction of individual crystallites tended to mask any changes of conductivity in their *c*-direction, unless these increases also happened to be large. Estimates of *c*-axis changes based on experiments with such material were thus not completely reliable.

Fairly thick pieces of stress annealed well parallelized graphite can, however, now be made in various ways⁶. Some pieces of commercially available well parallelized graphite kindly provided by Union Carbide (US) have recently been tested here. Using determinations of the intercalation threshold for nitric acid vapour⁷ the Union Carbide product proved to be similar to our own well parallelized graphite (stress annealed *in situ*) with respect to freedom from pinning and similar defects. These advances have eased technical difficulties in making measurements in the direction of the *c*-axis of synthetic metals after intercalating potassium atoms in donor layers or acid bisulphate, acid nitrate, bromine or iodine monochloride in acceptor layers.

Fig. 1 illustrates the mounting of a specimen for potentiometric *c*-axis measurements. Layers parallel to the basal planes have high conductivities, and serve as equipotential surfaces in what is in effect a four terminal mounting. As intercalation of any species proceeded in a piece of well aligned graphite, changes in the electrical conduction were recorded continuously using a Multipoint recorder with a microvolt preamplifier. The progress of the intercalation was regulated by controlling the thermodynamic chemical potential, in the case of all but the acid sulphates for which electrochemical control was used⁸. Control of the chemical potential was achieved either by controlling the temperature and the vapour pressure of the

molecules, for vapour phase intercalation, or by controlling the temperature and the concentration of molecules in an inert solvent, for example, when intercalation took place from a solution of halogen in carbon tetrachloride. The swelling of the specimen during intercalation could also be followed by optical microscopy; in some cases the weight uptake at different chemical potentials of the intercalating species was also measured independently using a quartz spiral spring balance⁷. Because of the high degree of parallelism of *c*-axes of graphite crystallites in the material used, swelling took place smoothly, and mechanical breakages were only a small problem. (Data in the direction of the *a*-axis were obtained with conductors mounted as described previously⁸.)

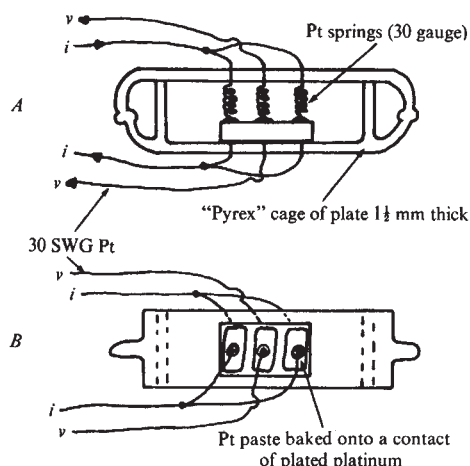


Fig. 1 The *c*-axis conductivity frame. A, Side view; B, top view.

With these experimental arrangements, I find that at 295 K, the *c*-axis conductivity of well aligned graphite showed considerable decreases on intercalation of the acceptor molecule Br₂, and of the anion complexes NO₃⁻, HNO₃ and HSO₄⁻ 2H₂SO₄ (Table 1). Intercalation of ICl made little difference to the *c*-axis conductivity of the parent graphite. Only the intercalation of potassium atoms caused a considerable increase of *c*-axis conduction. Data recorded in Table 1 refer to the intercalation stage specified, which was generally the limit attainable. Conductivities were calculated from measured resistances and dimensions of the specimens, allowing for the expansion in the *c*-axis direction as also indicated in Table 1.

Table 1 Conductivities (ohm⁻¹cm⁻¹) of Synthetic Metals in the *c*-Axis Direction

Conductor	295 K	(σ_{77}/σ_{295}) _c	Spacing between filled carbon hexagon sheets (Å)
Parent graphite	11.47	0.44	3.35
C ₈ K	3,220	3.48	5.4
C ₈ Br	1.77	4.74	7.05
C ₃₄ HSO ₄ 2H ₂ SO ₄	0.66	5.21	7.98
C ₄₈ NO ₃ 3HNO ₃	0.41	1.73	7.82
C ₈ ICl (estimated)	(16.3)	(1.87)	7.24

Conductivities in the *c* direction were also measured at 77 K by immersing the sample in boiling nitrogen. It is well known that the parent graphite shows a small decrease of conductivity in this crystal direction when cooled (Table 1). All the synthetic metals studied in this work showed an increase of conductivity

as their temperature was lowered, suggesting that scattering mechanisms in this direction are controlled by thermal vibrations of charged units in the crystals; this also seems to be the case in the *a*-axis direction³. Conductivity ratios (σ_{77}/σ_{295})_c are also recorded in Table 1 and illustrate the observed trend.

Even from this preliminary survey of *c*-axis conduction of these synthetic metals, it is clear that the *c*-*a* anisotropy of electrical properties of all the p metals measured so far is more pronounced than in the parent graphite, which is already a very unusual crystal in this respect. This can be seen from Table 2.

Table 2 Anisotropy Ratios of Electrical Conductivity (σ_a/σ_c) of Synthetic Metals Based on Graphite

Conductor	295 K	77 K
Parent graphite	2.5×10^3	10.3×10^3
C ₈ K	0.037×10^3	0.113×10^3
C ₃₄ HSO ₄ 2H ₂ SO ₄	300×10^3	229×10^3
C ₄₈ NO ₃ 3HNO ₃	600×10^3	$2,190 \times 10^3$
C ₈ ICl (estimated)	2.5×10^3	—

(Values of σ_a for parent graphite obtained from Union Carbide (see text).)

These high anisotropy ratios suggest that the flow of the electric charges in many of the crystals cited in Table 2 must be highly "layered", indeed almost two dimensional, in stacked conduction sheets separated by very poorly conducting material.

Unusual electromagnetic phenomena may be expected in conductors with highly layered structure. Certain types of "negative resistance", for example, in sulphides with a layer crystal structure such as SnS₂ and ZrSe₂, appear to be associated with layered current flow^{9,10} and "negative resistance" effects have been observed by us in various synthetic metals based on graphite. These will be described in a later publication. As the *a*-axis resistivities of the present group of conductors are much lower than for the semiconductors cited above, contact effects at the junctions can, however, play a predominant role. Interpretation of the unusual negative effects that are found with graphite intercalates still needs to be treated with caution, in view of diverse physical origins which might account for the phenomena observed.

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BIOLOGICAL SCIENCES

Polymers Formed by some β -Lactam Antibiotics

THE β -lactam antibiotics are the least toxic of all drugs, their major side effects being caused by hypersensitivity reactions of the type most commonly mediated by materials of relatively high molecular weight. It is generally thought that immunological reactions to β -lactam antibiotics are due to their ability to form covalent links with protein to produce material with the necessary high molecular weight. But the reaction between protein and antibiotic might be expected to be relatively slow and it is therefore difficult to explain, in these terms, anaphylactic reactions which, although rare, can take place within seconds of injecting the antibiotic. Autologous proteins to which foreign molecules are attached are usually only weakly immunogenic in comparison with the same material attached to a foreign protein. The finding that β -lactam antibiotics could contain preformed macromolecular contaminants was therefore of considerable significance^{1,2}. It was realized that these contaminants were of two types, one being proteinaceous and the other polymeric material derived from the antibiotic itself. The properties of penicillin protein conjugates have been the subject of many studies. The properties of polymers derived from the β -lactam antibiotics are less clearly understood. We have attempted to isolate polymers formed in aqueous solutions of some β -lactam antibiotics so as to study their chemical properties. The immunological properties of these materials are being investigated and the results will be reported separately.

The molecular weights of sodium salts of 6-aminopenicillanic acid (6-APA) and of ampicillin increase during storage as aqueous solutions³. This finding, together with the concomitant loss of primary amino-groups and β -lactam rings, led to the suggestion that polymers had formed with structures I and II (Fig. 1) but that they had intact terminal β -lactam rings. A dimer of 6-APA with a similar suggested structure has also been isolated from 1 week old solution⁴. Materials of high molecular weight have been isolated from solutions of 6-APA by Stewart⁵ who suggested that the relationship, if any, of these macromolecules to the structures I and II required further investigations.

It is perhaps not difficult to imagine polymers forming in solutions of materials containing both a free amino-group and a β -lactam ring susceptible to nucleophilic attack. Polymers, however, also form in solutions of β -lactam antibiotics not containing a free amino-group. It has recently been suggested⁶ that polymerization of benzylpenicillin might first involve β -lactam hydrolysis to give benzylpenicilloic acid.

This would cause a decrease in pH to conditions favouring the rearrangement of other molecules of benzylpenicillin to form benzylpenicillenic acid which, being a powerful acylating agent, would be capable of penicilloylating exposed thiazolidine nitrogens. This series of reactions would produce polymeric materials of structure III (Fig. 1).

We have attempted to isolate polymeric materials formed in solutions of 6-APA, benzylpenicillin, ampicillin and hetacillin. The benzylpenicillin was 'Purapen' which is a commercially available material known to be free of protein. The 6-APA was purified by adsorption techniques so as to be free of protein⁷ and the ampicillin and hetacillin were prepared from it.

Aqueous solutions (25% w/v) of the sodium salts of the antibiotics were stored in sterile conditions in the dark for 14 days. As the solutions aged, samples eluted with 0.5% brine through 'Sephadex G. 25 f' were found to contain increasing amounts of ultraviolet absorbing material in the high molecular weight region of the eluate. After 14 days there was

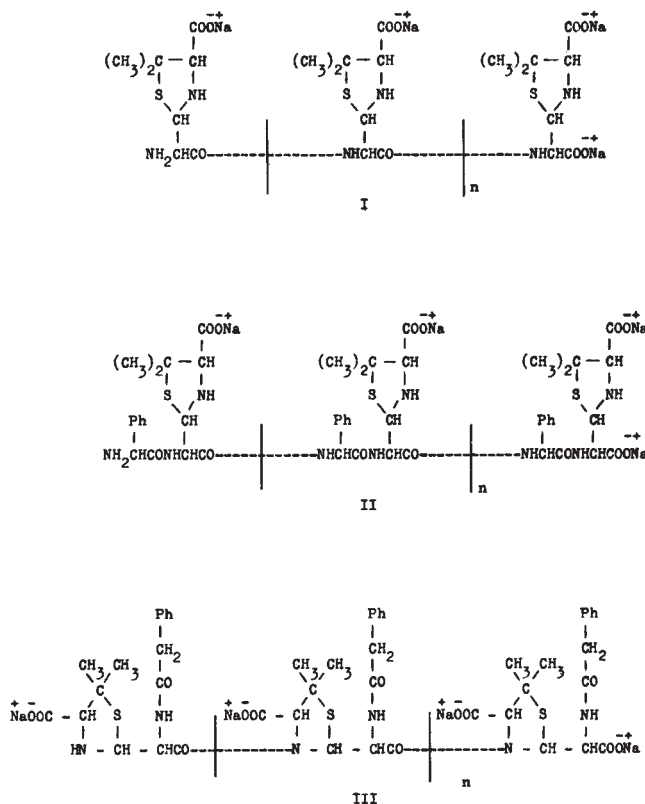


Fig. 1 Polymers of the sodium salts of 6-aminopenicillanic acid (I), ampicillin (II) and benzylpenicillenic acid (III).

little further change in elution patterns indicating that polymerization was complete and at this time material had just begun to appear at the void volume (K_{av} 0). After 14 days the pH of the solutions was found to have decreased to 5 for 6-APA and benzylpenicillin and to 7 for ampicillin and hetacillin. They were readjusted to the pH of the original solutions of the sodium salts. High molecular weight material was separated by repeated chromatography through 'Sephadex (G. 25 f)' first through a large column eluted with water to give a crude separation, then through a smaller column eluted with 0.5% brine to give good definition, and finally desalted by running through a small column eluted with water. The high molecular weight fractions (from K_{av} -0.1 to K_{av} 0.5 for the columns eluted with water and from K_{av} -0.1 to K_{av} 0.3 for the column eluted with brine) were separated after each run through the columns, adjusted to the pH of the sodium salt of the original antibiotic solution and freeze dried. Benzylpenicillin gave an overall yield from its original sodium salt of 0.8%, ampicillin 1.6%, hetacillin 0.7% and 6-APA 3.4%.

The results of investigations are shown in the Table 1. None of the polymers in solution showed a peak of absorbance at 322 nm, indicating little penicillenic acid content. The molecular weights of the polymers were determined from ultracentrifuge measurements of sedimentation and diffusion coefficients. These, together with values for partial specific volumes, were substituted in the Svedberg equation. The degree of polymerization was obtained by dividing these molecular weights by the molecular weight of the monomer. The assumption made was that the number average molecular weight would be close to the weight average molecular weight. For the polymers derived from ampicillin and 6-APA, measurements of the number of non-acylated thiazolidine rings and the β -lactam contents indicate that the non-amino terminal polymer units can contain an intact or hydrolysed β -lactam ring. Within the limits of the methods the assays provide support for the suggested structures I, II, III.

Table 1 Investigation of the β -Lactam Antibiotic Polymers

Investigation	Parent β -lactam antibiotic		
	Na benzylpenicillin	Na ampicillin	Na 6-APA
Elemental analysis	As for Na benzylpenicillin containing 16% H ₂ O	As for Na ampicillin 1½ H ₂ O	As for Na 6-APA 1 H ₂ O
Degree of polymerization	3.5	6.1	4.7
Primary NH ₂ ^a	None	0.99 per 6.1 U	0.88 per 4.7 U
Non-acylated thiazolidine rings ⁹	0.82 per 3.5 U	5.33 per 6.1 U	3.5 per 4.7 U
Intact β -lactam *	0.07 per 3.5 U	0.2 per 6.1 U	0.4 per 4.7 U
Free SH ¹⁰	0.04 per 3.5 U	0.06 per 6.1 U	0.3 per 4.7 U
Absorbance at 285 nm to 295 nm. No. of units in penamaldate form	1 in every 59.5 U	1 in every 100 U	1 in every 39.6 U
NMR aromatic proton gel dimethyl ratio	5 to 6	5 to 6	—
Antibacterial activity. <i>B. subtilis</i>	None detected	None detected	None detected

* Unpublished method.

The hetacillin polymer seemed to have the same structure as the ampicillin polymer. This is not surprising since hetacillin in aqueous solution rapidly hydrolyses to give ampicillin.

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Structure of β -D-(1 $\rightarrow$ 4')Xylan Hydrate

XYLAN is one of the principal hemicelluloses which may constitute up to 35% of the dry weight of hardwoods¹. It is composed of a backbone of β -(1 $\rightarrow$ 4')-linked anhydro-D-xylose units with occasionally attached L-arabinose and 4-O-methylglucuronopyranosyl side groups.

Polarized infrared investigations² of oriented films of xylan hydrate have indicated the presence of a weak (O₅ . . . O₃) intramolecular hydrogen bond. X-ray examination^{2,3} showed a fibre repeat of about 15 Å and the presence of only those meridionals for which $l=3n$ (where n is an integer); this demonstrated that the true axial repeat is one third of the

fibre repeat and strongly suggested that the xylan chain incorporates a three-fold screw axis.

Conformational analysis of xylan⁴ favoured a left handed helix, for which an intramolecular hydrogen bond (O₅ . . . O₃) is possible. Subsequent energy calculations⁵ showed that the minimum energy position was within 0.3 kcalories mol⁻¹ per residue of the most probable lattice conformation.

The xylan hydrate X-ray reflexion spacings quoted in the literature vary considerably; for example, the first equatorial d -spacing seems to vary between 7.76 Å (ref. 6) and 8.15 Å (ref. 7). It seemed probable that the cell parameters were a function of the water content. Good fibre orientation in the specimen of white birch xylan hydrate (prepared as described in ref. 3) enabled us to obtain a clear resolution of the reflexions on the equator and first layer line, the spacings indexing on a unit cell of hexagonal shape with dimensions:

$$a=b=9.16 \text{ Å}; c (\text{fibre axis})=14.85 \text{ Å}; \gamma=120^\circ$$

Increasing the relative humidity of the sample in stages increased the number of observed reflexions and decreased the exposure time; this is consistent with an increase in both the lateral order and degree of crystallinity of the sample. Meanwhile, an increase in the equatorial spacings was observed together with some marked changes in reflexion intensities, particularly on the equator and first layer line. The reflexions observed from the sample at 100% relative humidity index on a unit cell of dimensions

$$a=b=9.64 \text{ Å}; c (\text{fibre axis})=15.00 \text{ Å}; \gamma=120^\circ$$

When the xylan hydrate specimen was dried *in vacuo* for 2 days at 40° C, and the X-ray diffraction photograph taken with a camera evacuated and dried with calcium chloride, the diagram obtained gave spacings corresponding to the dry, poorly crystalline xylan of Marchessault and Timell⁶.

Although there is a continuous range of hydrated xylan structures, it is convenient to refer to the structure which has a spacings index on the unit cell of $a=b=9.16 \text{ Å}$, $c=14.85 \text{ Å}$ and $\gamma=120^\circ$, as xylan hydrate. Interest centred on this form because it contains the fewest water molecules commensurate with a good X-ray fibre diagram (fifteen observed reflexions) and was therefore the structure most susceptible to solution by the methods which will be outlined here.

As shown previously³, the bulk density of xylan hydrate clearly indicates that there are only six monomer units in the unit cell. The fibre repeat of nearly 15 Å corresponds to three monomer units (compare the cellulose two monomer repeat of 10.3 Å). Therefore, there are two chains per unit cell. In such a cell, the sole explanation of the presence of only those meridionals with $l=3n$ is if the xylan chain itself incorporates a three-fold screw axis. The X-ray fibre diagram does not indicate any symmetry relationship between the chains. Therefore, the second chain was positioned centrally between the four-corner chains. (Xylan chain coordinates corresponding to a three-fold axis with an advance per monomer of 4.95 Å were supplied by Dr P. R. Sundararajan.) It was assumed that the isolated chain conformation of minimum energy would be very similar to that taken up by the xylan chains within the lattice. To specify the structure completely, it is necessary to determine the relative rotations and translations of the chains and to locate the water molecules. A chain packing computer program was used to obtain the independent rotations to the central and corner chains and translate the central chain.

At each instance, all the non-bonded interactions were computed and compared with the contact distance criterion⁸. Neither parallel nor antiparallel systems were permissible on this basis and a re-investigation of possible chain positions (bearing in mind the observed structure factors for equatorial reflexions) suggested the sites shown in Fig. 1. Packing of the central chain in the antiparallel system (suggested by the occurrence of folded-chain crystals⁹) with its three nearest neighbours permitted two sets of rotation angles, one of which

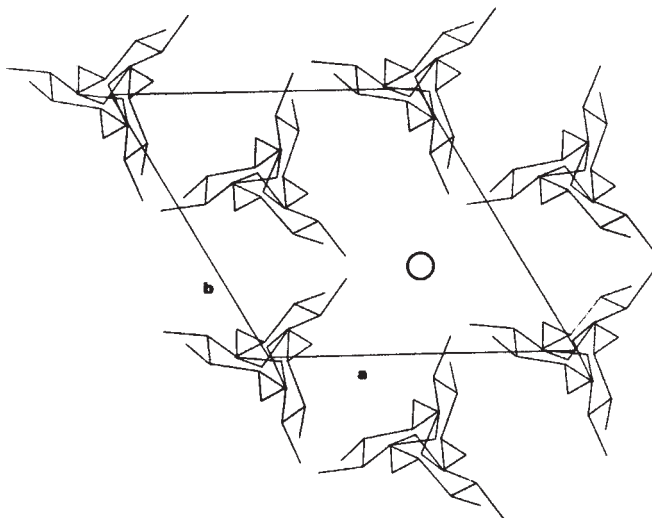


Fig. 1 Diagram of the proposed unit cell of xylan hydrate; projection down the fibre axis. The circle represents the position of the water column.

was eliminated because of its poor intensity fit. The other possible structure yielded acceptable intensity values. In this structure (Fig. 1) the unoccupied lattice position (2/3, 1/3) is clearly richly endowed with hydrophilic groups so that this would be the location of the water molecules. The structure factors for each of the equatorial reflexions were calculated for three, six, nine and twelve water molecules, successively. Subsequent attempts to refine the water molecule positions by model building were limited by the small number of observed reflexions and the numerous possible hydrogen bonding schemes. But intensity calculations for the equatorial reflexions indicate that the water molecules are unlikely to lie more than 0.5 Å from the lattice site, so that the water molecules probably form a column. Although, from packing considerations, both parallel and antiparallel systems are permissible, the latter is preferred on the basis of its agreement with the observed X-ray intensities.

The proposed structure for xylan hydrate corresponds to the $P3_2$ space group, the trigonal symmetry of which explains the retention of a hexagonal unit cell shape, irrespective of water content. The "empty" lattice site is able to accommodate water and also such side groups as 4-O-methyl glucuronic acid which occur on about one xylose group in ten. Unlike cellulose¹⁰ and mannan¹¹, xylan is unable to form strong intermolecular hydrogen bonds and seems to depend on the presence of water to stabilize the structure.

Clearly, the uptake of water by xylan is important both biologically in the water economy of plants, and commercially in the fabrication of paper.

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Is the Inhibition by Cycloheximide of Induced Long Term Changes in Cortical Activity due to Inhibition of Protein Synthesis?

I WISH to report further evidence that protein synthesis is necessary for the production of long term increases in cortical neuronal activity. Although this change has been referred to as "after-effect", to avoid confusion I call it long term increase (LTI).

LTIs, which have been described in detail already^{1,2}, may be elicited by many forms of stimulation which elevate the spontaneous discharge rate of cortical neurones (for example, passage of direct current for 10 min or more between an anode on the cortex and an indifferent electrode elsewhere on the body) in a rat anaesthetized with 'Urethane'. After stimulation a persistent (more than 6 h) elevation of cortical neuronal discharge rate is observed. I have discussed a possible mechanism for the persistence of these LTIs^{3,4}.

I found that topical application of the protein synthesis inhibitors cycloheximide, chloramphenicol and neomycin to the cortex before stimulation did not prevent an increased discharge rate during stimulation, but prevented the development of the usual after-effects of this stimulation. It would be an important indicator that these drugs prevent LTIs by inhibiting protein synthesis if they had a similar dose response curve in these actions. I therefore carried out parallel biochemical studies using cycloheximide as the inhibitor. The electrophysiological experiments have already been described⁴ and so I shall report only the biochemical studies and correlations here.

Rats were anaesthetized with 36% 'Urethane' (1.8 g/kg) and one trephine hole was made² over each hemisphere. The dura was reflected and a polythene ring was cemented over each hole to form water tight cups. The rats were then placed, four per experiment, in an enamelled tray and covered with paper tissues to keep them at a steady rectal temperature of 35°C.

Drug or saline (0.1 ml.) was placed in each cup and left for 30 min to soak into the cortex. This solution was then removed and 0.1 ml. of a solution containing 0.5 µCi of ¹⁴C-U-1-leucine (Radiochemical Centre, Amersham, CFB67, diluted ten times with 0.9% saline) was placed in each cup. The rats were then left for 1 h for some incorporation to take place and then killed. The cortex was removed rapidly with the aid of a sharp edged, thin walled stainless steel tube with a 4 mm outside diameter. This operation removed a core of brain, and the top 1.5 mm was separated off with a spatula, and homogenized in 1 ml. of distilled water. The protein was precipitated with 6 ml. of 12% trichloroacetic acid (TCA) and left to stand for 10 min. The protein was separated using a 'Millipore' filter and a tared Whatman GFA glass fibre disk. The crude protein was washed once with 12% TCA, once with water, three times with acetone, three times with ether and dried in hot air at 50°C. The disk was reweighed to ascertain the weight of protein, and placed in a counting vial with 2 ml. of scintillant and counted in a Beckman CPM100 scintillation counter using a wide ¹⁴C window. Also a 0.1 ml. sample of the original filtrate was placed on a glass fibre disk and counted by the same method.

After allowing for background counts results were expressed as c.p.m./mg of protein 10^6 counts in the filtrate. At least four determinations were performed at each dose of inhibitor. Using data obtained from control experiments with no inhibitor, results were expressed as percentage inhibition of protein synthesis.

Table 1 Effects of Various Doses of Cycloheximide on the Inhibition of LTIs and on the Inhibition of Protein Synthesis

Cycloheximide dose (g/l.)	Inhibition of LTI (%)	Inhibition of protein synthesis (%)
0.0001	14	30
0.001	50	10
0.01	100	67
0.1	86	76
1.0	100	89

The results are presented in Table 1, with values for the percentage inhibition of the LTI for each drug taken from ref. 4.

The reliability of correlation of inhibition of protein synthesis with the logarithm of the dose of cycloheximide was tested using a Kendal rank correlation test⁵, which gave a significant correlation ($S=8$, $N=5$, $P<0.042$). The same test was used (allowing for ties) for the correlation of inhibition of LTI with the logarithm of cycloheximide dose. This also gave a significant degree of correlation ($S=7$, $N=5$, $P<0.042$).

To test whether inhibition of protein synthesis by cycloheximide blocks the LTI produced by anodal cortical polarization, I compared the dose response curves for both effects using Kendal's concordance test, which compares the reliability of several different measures on sets of data, to show whether the measures are using the same standards of judgment. This test gave the following results. With $N=5$ and $K=3$ then $W=0.86$ and $S=77.5$ which gives $P<0.01$. Thus there is a strong correlation between drug dose, inhibition of protein synthesis and the inhibition of LTIs, and so it is likely that LTIs are inhibited through an inhibition of protein synthesis.

Most work on inhibitors of protein synthesis has been performed on cell-free or other relatively simple preparations, and so experiments involving the use of such drugs must be performed with care in the whole animal because side effects may be responsible for any effects seen. For example, intra-cerebral injection of puromycin⁶ gives rise to epileptiform activity in the hippocampal electroencephalogram. Actinomycin D, an inhibitor of RNA synthesis, in doses which have little effect on RNA synthesis, produces cellular damage and abnormal electrical activity in the hippocampus⁷.

It is therefore necessary, in studies involving protein synthesis inhibitors in physiological and behavioural situations, to check that protein synthesis inhibition has a similar dose response curve to that of inhibition of the effect under investigation. There is evidence⁴ that drugs which block protein synthesis, for example, cycloheximide, chloramphenicol, neomycin B and tetracycline, have no effects on spontaneous activity or induced activity of the cortex although all prevent LTIs.

This evidence, together with that presented here, strongly suggests that protein synthesis is essential for the establishment of LTIs. The final test of this hypothesis will be to investigate whether the establishment of LTIs is accompanied by an elevation of cortical protein synthesis rate, and I hope to do this soon.

I thank Action for the Crippled Child for a grant and Dr H. Davson for allowing me to use his scintillation counter.

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Persistence of Morphine in Central Nervous System of Rats after a Single Injection and its Bearing on Tolerance

TOLERANCE of a very high grade and specificity is known to develop to morphine and its congeners. Although no precise information is available on the biochemical and anatomical sites of morphine-receptor interaction, it is generally believed that the matrix of the central nervous system is directly involved in many parameters of the response due to morphine-like analgesics. Morphine, however, has no special affinity for the central nervous system and only minute amounts are needed in brain to elicit the typical pharmacologic effects. Further, morphine concentrations in brain have been shown^{1,2} not to be necessarily related to the analgesic effect. Failure to demonstrate changes in the rate of excretion, distribution, increased metabolic degradation or transformation of morphine³⁻⁵ has suggested biochemical alteration of specific neural structures in the development of tolerance. Intracellular distribution studies^{6,7} with morphine administered *in vivo* showed it to be localized primarily in the soluble microsomal fraction of rat brain homogenate either in the free or lightly bound form and 10-25% of drug in crude nuclear brain fraction. This distribution pattern did not change with the development of tolerance. Tolerance has also been shown⁸ to develop apparently at the very first dose administered and some degree of tolerance in rats lasting as long as a year resulted from a single injection of morphine⁹. These observations are difficult to explain in view of the known disappearance of free morphine from the brain within 48 h of a single injection. All the previous studies, however, have centred chiefly on the presence of free drug in the central nervous system. This study provides evidence that at all periods of time up to 3 weeks or more (periods longer than 3 weeks not investigated) after a single subcutaneous injection of 10 mg/kg of ¹⁴C-morphine-N-methyl (synthesized by a method previously described¹⁰ and its chemical and radiochemical purity established by thin-layer chromatography in different solvent systems) in the rat there was a consistently higher radioactivity present in brain than could be accounted for on the basis of free morphine alone. Measurable and significant amounts of drug were present as polar "conjugated" drug and the ratio of "conjugated" morphine to total radioactivity increased with time up to 24 h. Thereafter all the radioactivity was wholly due to "conjugate". Further, radioactivity amounting to 10-20 ng/g of brain (approximately $2-4 \times 10^{13}$ molecules) could be detected in brain 3 weeks after a single subcutaneous injection of 10 mg/kg of morphine. Up to 2 h after injection of the drug, part of the radioactivity due to "conjugate" could be acid-hydrolysed to free morphine but at later times the "conjugate" was not hydrolysable with dilute acid. The nature of this conjugation of morphine and its possible bearing on the development of tolerance is discussed.

Male Sprague-Dawley rats weighing 100-150 g were injected subcutaneously with 10 mg/kg of ¹⁴C-morphine-N-methyl (specific activity 1.1 μ Ci/mg) and decapitated at different periods of time, the cerebral cortical hemispheres removed, wiped of adhering blood by soft tissue paper, rinsed in ice-cold saline, rewiped and weighed. Free morphine was determined

Table 1 Uptake of ^{14}C -Morphine-N-methyl by Rat Cerebral Cortical Hemispheres at Different Times after a Single Subcutaneous Injection of 10 mg/kg*

	30 min	60 min	2 h	4 h	6 h	12 h	24 h	7 days	14 days	21 days
Total radioactivity	483	474	214	137	94	27	21	12	12	18
Free morphine	324	285	125	30	13	8	7	—	—	—
Free+ acid-hydrolysable morphine	407	335	153	33	36	7	4	—	—	—
Total bound morphine	159	189	89	107	81	19	14	12	12	18
Ratio of total bound/total radioactivity	0.33	0.40	0.42	0.78	0.86	0.70	0.67	1	1	1
Ratio of non-acid hydrolysable bound/ total radioactivity	0.16	0.29	0.29	0.76	0.62	0.70	0.67	1	1	1

* Data represent the mean value (ng/g wet brain weight) of two animals for each determination of total radioactivity, free morphine and free plus acid-hydrolysable conjugate.

by modification of procedure⁴, by homogenization of the brain in 0.5 N HCl, basification to pH 9, buffering with 40% K_2HPO_4 solution, extraction with ethylene dichloride containing 30% by volume of *n*-amyl alcohol by shaking for 30 min, centrifugation and washing of the organic phase with 4% K_2HPO_4 solution. Aliquots of organic phase evaporated in counting vials were counted in a liquid scintillation counter as before⁴ and counts were corrected for quenching by addition of ^{14}C -toluene as internal standard. Samples of brain homogenate were hydrolysed with 20% by volume of concentrated hydrochloric acid by autoclaving at 15–20 pounds pressure for 1 h, adjustment to pH 9 and extracting for free morphine and that liberated from acid-hydrolysable "conjugate". Total radioactivity was determined by dissolving the brain samples

(1–1.2 g) in 4 ml. of NCS (a quaternary ammonium hydroxide obtained as a 0.6 N solution in toluene from Amersham/Searle Corp., Des Plaines, Illinois) in capped counting vials by digestion on a Fisher-Slide warmer at 45° C for approximately 48 h with occasional shaking to break the lumps, adding phosphor-toluene solution, thoroughly cooling the samples, counting and correcting for quenching using ^{14}C -toluene as the internal standard. Brains of rats not injected with drug were similarly dissolved in NCS and counted to serve as backgrounds for the total radioactivity determinations. The mean values on the uptake of morphine by rat cerebral cortical hemispheres at different times after a single subcutaneous injection of 10 mg/kg of ^{14}C -morphine-N-methyl are given in Table 1.

For the qualitative identification of drug and its polar "conjugate", six brains removed 4 h after an injection of 10 mg/kg of ^{14}C -morphine-N-methyl were homogenized in 2 N ammonium hydroxide, extracted repeatedly with a mixture of chloroform-isopropanol (1 : 1) and organic extracts removed by centrifugation. Aqueous layer and brain debris were treated with ethanolic 1 N ammonia repeatedly. Residues from pooled organic and ethanolic ammonia phases by evaporation under reduced pressure subjected to thin-layer chromatography on Gelman instant thin-layer chromatography media silica gel (ITLC) (Fig. 1) showed the presence of morphine R_F 0.87, and "conjugate" R_F 0.26, with solvent system *n*-butanol : acetic acid : water (35 : 3 : 10 v/v) and confirmed by further chromatography in solvent system *n*-butanol : *n*-butyl ether : concentrated ammonia (25 : 70 : 2 v/v) (R_F morphine 0.56; R_F "conjugate" 0.0) and ethyl acetate : methanol : concentrated ammonia (17 : 2 : 1 v/v) (R_F morphine 0.90). Similar results were obtained when brains were homogenized in saline or 0.5 N HCl and processed as given above. These chromatographic experiments established the identity of the free drug as morphine and specifically ruled out the presence in brain of morphine N-oxide or codeine. On chromatographic evidence¹¹ the acid-hydrolysable "conjugate" seems to be morphine-3-glucuronide. Brain debris, dried *in vacuo*, autoclaved in 10% by volume of concentrated HCl for 1 h at 15–20 lb pressure, acid supernatant centrifuged, evaporated under nitrogen, adjusted to pH 7 and thin-layer chromatographed using solvent system *n*-butanol : *n*-butyl ether : concentrated ammonia (25 : 70 : 2 v/v), showed one peak of radioactivity due to nonhydrolysable "conjugate" (R_F 0.0).

Prolonged, firm and persistent association of radioactivity in brain suggests a firm attachment or bonding of morphine or more probably its metabolite with some specific intracellular constituent such as protein or nucleic acid. Binding of morphine, however, *in vitro* to brain⁶ and serum proteins¹² is of weak electrostatic type and easily ruptured by prolonged dialysis or dilute acid or alkali treatment. Physico-chemical evidence¹³ has been produced to support the fact that morphine and its congeners possessing a free 3-phenolic group form highly polar water-soluble 2 : 3 *o*-quinones in ammoniacal conditions in the presence of dilute H_2O_2 and trace amounts of copper as catalyst at ambient temperature (Fig. 2). Compounds with a masked phenolic group did not give this reaction. Firm

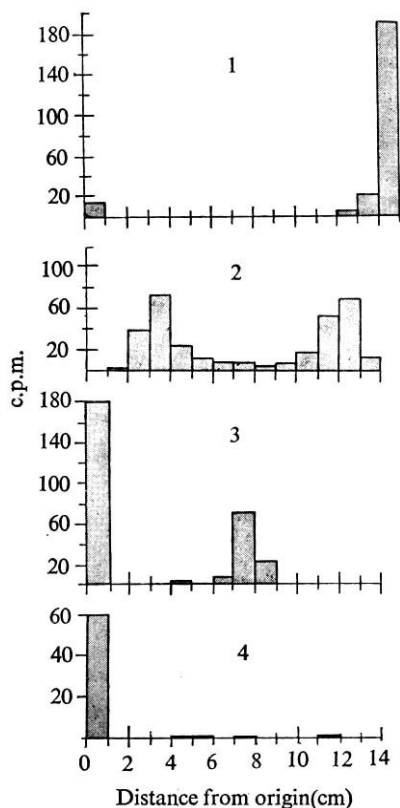


Fig. 1 Radioscans of thin-layer chromatograms (ITLC) of (1) organic extract of brains of rats removed 4 h after a subcutaneous injection of 10 mg/kg of ^{14}C -morphine-N-methyl, ascending technique using solvent system ethyl acetate:methanol:concentrated ammonia (17:2:1 v/v); (2) organic and aqueous ethanolic extract of brains using solvent system *n*-butanol:acetic acid:water (35:3:10 v/v); (3) same as (2) run in solvent system *n*-butanol:*n*-butyl ether:concentrated ammonia (25:70:2 v/v); (4) acid-autoclaved brain debris extract neutralized to pH 7 using solvent system *n*-butanol:*n*-butyl ether:concentrated ammonia (25:70:2 v/v).

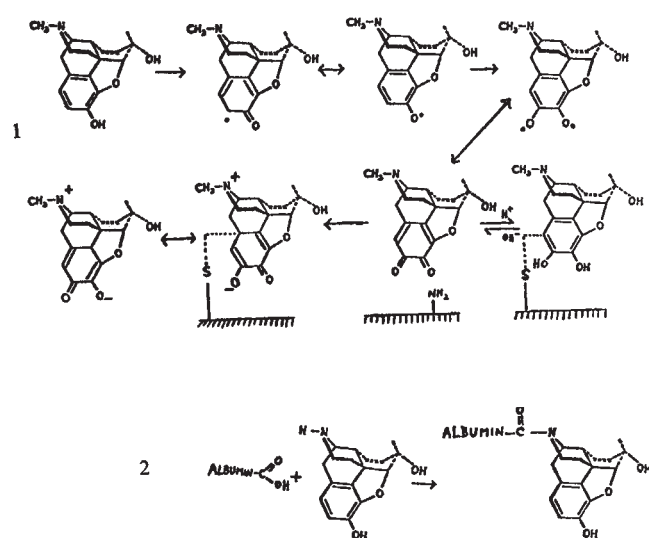


Fig. 2 1, Outline of scheme for oxidation of morphine in ammoniacal H_2O_2 solution in presence of trace amounts of copper as catalyst to generate the corresponding 2 : 3-catecholyl type derivative or corresponding zwitterionic *o*-quinone and probable sites of covalent conjugation with intracellular protein sulphhydryl or amino-groups; 2, covalent conjugation of normorphine with human serum albumin in presence of a substituted carbodiimide.

coupling by covalent conjugation of morphine with specific brain proteins at an appropriate neuronal site could take place through intermediate formation of a catechol or *o*-quinone type of derivative through the catalytic effect of copper ions under ammoniacal conditions (Fig. 2) as copper is known to be concentrated several-fold relative to other metals in nerve endings, synaptosomes and synaptic vesicles of brain tissue. Ternary coordination complexes of catecholamines¹⁴ and 5-hydroxytryptamine¹⁵, copper and ATP represent a mechanism for storage or intravesicular binding sites for these monoamines. Coupling interactions of enzyme-generated catechols (*o*-quinones) and amino or sulphhydryl groups of proteins to form N or S-catecholyl or quinonoid-protein conjugates have been shown¹⁶ to take place readily.

Two other possibilities of interaction of morphine with specific intracellular receptor protein of brain involving (a) addition of thiol peptides¹⁷ to 7 : 8 double bond through activation of 6-alcoholic group to a ketone and (b) conjugation of normorphine* through secondary amine group must be mentioned in view of the formation of normorphine as a metabolite *in vivo*¹⁸ and in rat brain¹⁹.

Finally, hapten-protein conjugates produced by irreversible binding of low molecular weight drugs or their degradation products to tissue proteins through covalent bonds have been shown²⁰⁻²⁴ to induce antibodies specific to such drugs which counteract their biological effect by concentrating their effect at one or a few sites or target organs and not acting against all the circulating drug.

Although the magnitude of differences in distribution of free ¹⁴C-morphine-N-methyl in the central nervous system of non-tolerant and tolerant animals was not adequate to account for high levels of tolerance observed^{4,5} it is conceivable that repeated administration of morphine could lead to cumulative deposition of "conjugate" in view of the long intracellular persistence of morphine or its polar metabolite. These observa-

tions are interesting in the light of recent evidence²⁶ that (a) biosynthesis of new RNA and *de novo* protein synthesis in brain is an essential feature of morphine tolerance in rats; (b) inhibitors of protein synthesis such as cycloheximide²⁷ or actinomycin D block the development of tolerance; and (c) a small dialysable protein or peptide from brains of morphine tolerant rats and dogs could confer tolerance in naive mice²⁸. This study does not indicate the drug-sensitive site of firm and persistent intracellular binding of morphine in brain but it is conceivable that such a "permanent" attachment of drug with target protein could bring about a biochemical alteration of specific neuronal structure in the central nervous system and produce hyperexcitability if the site happens to be one where a receptor neurotransmitter interaction occurs. 5-Hydroxytryptamine has recently been found to be in macromolecular association with specific proteins of hypothalamus²⁹. The catechol type of polar metabolite because of its close structural similarity to epinephrine could become an essential substrate in an alternative pathway of metabolism.

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* *In vitro* conjugation of normorphine (27 mg in 1 ml. dimethyl formamide) with human serum albumin (50 mg in 4 ml. water) performed by classical method²⁵ using 1-cyclohexyl-3-(2-morpholino-ethyl)-carbodiimide metho-*p*-toluenesulphonate (200 mg), prolonged shaking at ambient temperature and exhaustive dialysis against water and formation (R_f 0.0) established by thin layer chromatography with solvent system *n*-butanol : acetic acid : water (35 : 3 : 10 v/v) and polyacrylamide gel electrophoresis (pH 9.0).

Possible Clonal Origin of Common Warts (*Verruca vulgaris*)

FEMALES heterozygous for the X-linked glucose-6-phosphate dehydrogenase (G6PD) locus have two populations of cells, each expressing a single phenotype corresponding to one or the other of the two alleles¹⁻³. Where subjects are heterozygous for the A and B electrophoretic variants of G6PD⁴, one cell population will show only the A electrophoretic variant and the other only the B. The study of these variants has been used to throw light on the origin of certain tumours. Thus, uterine leiomyomas from Negro females heterozygous for G6PD variants A and B are of only one phenotype, although the adjacent uninvolved tissue shows both phenotypes⁵, which is consistent with a clonal or unicellular origin for this tumour. Similarly, chronic granulocytic leukaemia⁶ is probably of clonal origin, like the metastatic lesions from one patient with chronic lymphocytic leukaemia⁷. On the other hand, studies of metastatic lesions from a colonic malignancy⁷ and epidermoid carcinomas of the cervix have revealed⁸ the presence of both G6PD phenotypes, which implies a multiclonal origin.

We have used this technique for the study of verruca vulgaris or common warts—thickenings or projections of the epidermis, classified as benign tumours of the skin, caused by a spherical virus 50 µm in diameter⁹ which is structurally similar to the polyoma virus¹⁰.

Verrucal tissue was obtained in a dermatology clinic from Negro females. Lesions were diagnosed as typical verruca vulgaris by J. H. The study included only lesions with typical appearance¹¹, and measuring 2–8 mm in diameter. They were excised surgically without removing adjacent normal tissue. The root of the wart was destroyed by cryotherapy and the specimen placed in a sterile tube containing about 2 cc of TC 199 tissue culture medium. Venous blood was taken from the patient (5–10 cm³) and placed in heparinized, sterile containers and stored at 4° C until analysed (usually within 48 h). There was no significant loss of G6PD activity from the wart tissue in this time. Some samples were stored at –20° C until analysed. The G6PD phenotype of the red cells and corresponding wart specimens was determined on the same run. At the conclusion of each run the gel was sliced and stained¹² for zones of G6PD activity.

Only one specimen of verrucal tissue was obtainable from each individual, although if lesions were of sufficient size, they were cut into pieces and two or more runs made.

Adjacent normal skin tissue was not used to determine the patient's phenotype to minimize possible scarring after removal of the verrucae. Other evidence^{13,14}, however, indicates that the erythrocyte G6PD phenotype in heterozygotes is usually an adequate qualitative reflexion of the phenotype of skin or other tissues. In exceptional cases, the red cell phenotype is falsely homozygous although false homozygosity in skin in the presence of a heterozygous red cell phenotype has not been described. Thus, any resulting misclassification will probably result in a loss of information, but not misleading information.

Tissue and blood from twelve Afro-American females were tested. On starch gel electrophoresis wart tissue showed a single principal zone of G6PD activity and a slower minor zone. The former zone has the same mobility as that visible in mammalian liver and epidermal tissue and was found to have substrate specificity for galactose-6-phosphate dehydrogenase activity^{15,16}. The mobility of this zone did not vary with the phenotype of the wart tissue.

Six of twelve patients studied were heterozygous for G6PD electrophoretic variants as determined from their red blood cell phenotypes (Table 1). In two of the six heterozygotes, the phenotype of the wart tissue was A only and in four cases B only. The blood of the remaining six patients showed only a single phenotype (one A and five B). The G6PD phenotype in the wart and blood was the same in each case. Three large warts, one from a heterozygote and two from homozygotes,

Table 1 G6PD Phenotype of Blood and Wart Tissue of Twelve Afro-American Females determined by Starch Gel Electrophoresis

No.	G6PD phenotype, blood	No.	G6PD phenotype, wart
5	B+	5	B+
1	A+	1	A+
6	A+B+	4	B+
		2	A+

were each cut into four approximately equal pieces, but the phenotype was the same in each piece.

Verrucal tissue from individuals heterozygous for the common A and B electrophoretic variants of G6PD showed only one phenotype in each case tested, which is consistent with the hypothesis that verruca vulgaris is of clonal origin. This suggests that the verrucal virus infects a patch of cells all of the same G6PD phenotype, or that it infects only a single epidermal cell. The available data (six heterozygotes) are too limited to differentiate clearly between origin from a single cell or from a patch, especially as a normal patch size may be of the order of a thousand cells or more⁵. It is also possible that cells with both G6PD phenotypes are infected, but that one type is lost during cell proliferation.

Viral infection would be expected to involve a rather large target with spread to thousands of adjacent cells assuming a reasonable titre of the virus. If the proliferative process involves the spread of the virus, this should result in a lesion of multicellular origin, showing both G6PD phenotypes. On the other hand, if the process involves a change in a single cell or a small group of cells, what we have found would be expected, namely, evidence for single cell or clonal origin. The acidophilic inclusion bodies that are characteristic of pathologic sections of verruca vulgaris might still represent virus particles that have replicated with the proliferating cells rather than spread from an initial point of infection.

With the recent implication of viruses in the aetiology of some animal malignancies like the Rous sarcoma, and the murine leukaemias, and evidence that a tumour, like Burkitt's African lymphoma, is of possible clonal origin¹⁷ and may be caused by reovirus 3 (ref. 18), the behaviour of viruses in causing tumour-like lesions takes on considerable significance. The somatic mutation that has been hypothesized by many to lead to neoplastic change might, in some cases, be preceded by the viral invasion of a susceptible cell or clone of cells, or all cells may harbour in a symbiotic fashion this virus and a somatic mutation may induce its oncogenic properties.

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Possible Explanation for Loss of Detectable Antibody in Patients with Disseminated Malignant Melanoma

SINCE the demonstration of immunity to animal tumours¹ evidence has accumulated² indicating tumour specific antigens and corresponding antibodies in human malignant disease²⁻¹⁰. In malignant melanoma, antibodies have been detected only in patients with early or localized tumours^{11,12}. After progression from localized to disseminated melanoma, antibodies are not detected in the patient's sera¹¹. Attempts have been made to explain this disappearance of antibody in terms of a loss of recognizable antigenicity in the metastatic melanoma cells or antigenic modulation¹³, inability of the patient to continue producing an immune response to the melanoma; a disseminated tumour acting as an antigen "sponge" soaking up all available antibody, and a production of a "blocking" substance causing inactivation of the tumour specific antibodies.

The first two suggestions have been largely refuted by recent work, in which patients with disseminated malignant melanoma,

having no detectable antibody, responded to auto-immunization with their own tumour cells, and produced antibody in their sera¹⁴. The third possibility is considered unlikely in the light of lack of detectable antibody on the surface of cells freshly removed from patients with disseminated tumours, tested by direct immunofluorescence. We have also failed to detect antibody on melanoma cells, even when directly conjugated gamma globulin from autologous sera was used, after auto-immunization. The fourth possibility was therefore investigated.

Sera were obtained from three patients before and after auto-immunization with autologous irradiated melanoma cells as described previously¹⁴. In two of the cases the pre-immunization sera were found, by means of immunofluorescence using the membrane and the cytoplasmic techniques^{11,15}, to be negative. Serum from the third patient showed weak fluorescence (1:4) when the pre-immunization serum was tested against the autologous cells. In all three cases, strongly positive sera were obtained during the 7 days following auto-immunization (1:32-1:64) and these were selected for the following experiments, summarized in Table 1.

Membrane immunofluorescence^{11,15} was not obtained with the autologous positive sera (post-immunized) when it was first mixed with negative (pre-immunized) sera. The mixing reduced the fluorescence titres from 1:64 to 1:2 in two cases and from 1:32 to 1:4 in the third case. None of the control sera (AB), pooled human sera and heterologous negative melanoma sera altered the titres appreciably. When negative sera were added to the tumour cells before addition of positive sera, no clear blocking effect was demonstrated. In two cases, separated immunoglobulin from the negative sera was mixed with the positive autologous sera, and in both, the blocking effect was largely in the IgG fractions. Using cytoplasmic immunofluorescence^{11,15} with the above sera, no blocking could be demonstrated. In two cases single radial diffusion^{16,17} was seen between the negative gamma globulin¹⁹ fraction and positive gamma globulin fraction; it was not seen when AB or pooled human serum was used. Using double diffusion on cellulose acetate membrane^{20,21}, a precipitation line was seen between the positive serum up to a titre of 1:16 with the negative serum, but neither reacted with the controls. The separated immunoglobulins of the negative sera produced significant lines of reaction only between the IgG fraction of

Table 1 Summary of Results

Methods	Materials	Results
Membrane immunofluorescence (living cells)	Pre-immunized (-ve) sera. Post-immunized (+ve) sera. +ve sera conjugated to FITC for direct staining. FITC anti-human gamma globulin for indirect staining	(I) Blocking of +ve sera IF by -ve autologous sera only. No blocking by controls (II) IgG of -ve blocked autologous +ve sera (III) Blocking only seen when sera mixed prior to IF staining
Cytoplasmic immunofluorescence (fixed cells)	As above	No blocking effect demonstrated
Single radial diffusion	Cellulose acetate membrane (CAM) impregnated with +ve sera and 1 µl. of -ve and control sera added	Radial diffusion up to a titre of 1:16 between -ve and +ve sera
Double diffusion	5 µl. of +ve sera in centre of CAM-1 µl. doubling dilutions of -ve and controls around periphery. Repeated with immunoglobulin fractions	(I) Precipitation lines between +ve and up to 1:16 of -ve (II) Precipitation lines between -ve and up to 1:8 of +ve (III) IgG fraction of -ve reacted against +ve gamma globulin
Immunoelectrophoresis	Performed on CAM at pH 3.0, 7.2 and 10.0	Identical lines of reaction between +ve, anti-IgG and anti-human against the -ve IgG at all three pH values
Haemagglutination	Formalized sheep erythrocytes coated with either -ve or +ve gamma globulin	(I) Agglutination only between +ve and -ve (II) -ve action removed by absorption with +ve coated cells
Cytotoxicity	Cells grown in tissue culture and autologous sera and controls added	Action of +ve blocked by autologous -ve

In all these tests AB, pooled human sera and phosphate buffered saline were used as controls. FITC, Fluorescein-Isothiocyanate.

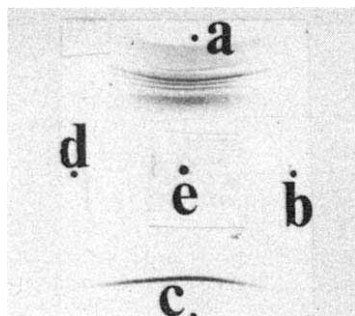


Fig. 1 Double diffusion on cellulose acetate membrane (CAM) showing a line of precipitation between the gamma globulin of the positive (post-immunized) and the IgG of the negative (pre-immunized) serum of melanoma patient 1. *a*, Anti-human serum; *b*, IgM of negative serum; *c*, IgG of negative serum; *d*, IgA of negative serum; *e*, gamma globulin of positive serum.

negative serum and the positive gamma globulin fraction (Fig. 1). This was repeated and also found to be identical, using highly purified IgG from a negative melanoma serum.

In immunoelectrophoretic studies^{20,21} (Fig. 2) a reaction was found only between the IgG fraction of the negative serum and both the positive gamma fraction and anti-IgG. The reaction between the anti-human serum and the IgG fraction of negative serum showed only one line, even after treatment at pH 3.0 or pH 10.0 (a treatment that normally causes dissociation of antibody-antigen complexes).

Formalized sheep erythrocytes coated with gamma globulin²³⁻²⁵ of the positive sera were agglutinated by addition of the gamma globulin fraction of negative sera. The non-gamma globulin fractions produced no effect. After agglutination had occurred and the red cells had been centrifuged, the supernatant no longer agglutinated freshly coated cells. In one case the positive serum produced a complement dependent cytotoxic effect^{9,26} on the autologous melanoma cells. This was considerably reduced by adding an equal volume of negative serum beforehand; a similar addition of pooled human or AB serum did not reduce the cytotoxicity.

The production of so called "blocking antibodies" in transplantation and tumour systems, causing immunological enhancement, has been suggested²⁷⁻³⁰ and recent experiments have shown that there is a prolongation of rat kidney grafts if antisera against the donor kidney are given to the recipients³¹. Blocking sera have been demonstrated in human tumours, where the effect seems to be directed against autologous lymphocytes^{32,33}, but the exact nature of the component in such sera is not known. The suggested explanation is that incomplete antibody reacts with the antigen on the target

cells, thus preventing the autologous lymphocyte from having a cytotoxic effect. The "protective" sera may be partly removed from the target cells and it would have relatively low avidity for the tumour specific transplantation antigens³².

However important cellular immune reactions may be in rejection of grafts or localized deposits of tumour, the possibility that humoral antibody plays a role in dealing with circulating tumour cells must be considered⁷. Such a function in malignant melanoma may have some beneficial value, particularly in delaying dissemination^{9,11,12}. Pre-immunized sera from disseminated melanoma patients have a direct blocking effect on the positive post-immunized sera raised in the same patient only a matter of days later. This effect is specific for the autologous positive serum and is contained in the IgG fraction of the negative serum. Even treatment of a highly purified preparation of this IgG fraction of negative serum, with buffer at pH 3.0 or at pH 10.0, still resulted in a line of precipitation with positive gamma fraction, anti-IgG and anti-human, the latter showing only one line of reaction. It is therefore more likely that a specific antibody against the original tumour specific antibody, rather than an antigen-antibody complex, is the "blocking agent". Such complexes with excess antigen could remain in solution³⁴ and be capable of blocking the positive serum, and could give a line of identity with anti-IgG, but treatment at pH 3.0 or pH 10.0 would be expected to cause dissociation of the complex. In addition, the mobility of such a complex would not be identical to pure IgG. In this respect, the phenomenon differs fundamentally from the blocking sera described in lymphocyte or colony inhibition studies in that it is directed not against the tumour antigen but against the tumour antibody itself. The effect in terms of the relationship between patient and tumour will of course be very similar, and this can also explain the progression of an antigenic tumour in a host with a normal intact immune system.

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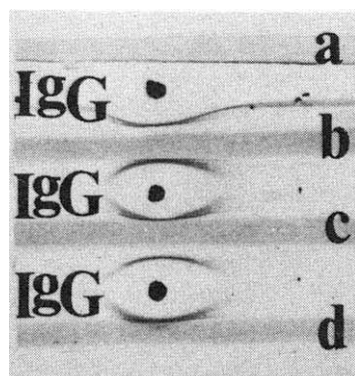


Fig. 2 Immunoelectrophoresis on CAM, showing a specific reaction between the IgG of the negative (pre-immunized) serum from melanoma patient 1 and its autologous positive (post-immunized), anti-IgG and anti-human sera. *a*, Gamma globulin of negative serum (melanoma patient 1); *b*, gamma globulin of positive serum (melanoma patient 1); *c*, anti-IgG serum; *d*, anti-human serum; IgG, IgG of negative serum.

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Asbestos and Mesothelioma in Man

RECENT international investigations into the relationship between exposure to asbestos dust and cancers have confirmed the paradox that, although there is a clear association between the development of diffuse mesotheliomas and exposure to crocidolite dust in the north-western Cape¹⁻², these tumours are very rare in the Transvaal where both crocidolite and the

closely related amosite are mined². Sluis-Cremer³ has reported that none of the factors which he examined (mineralogical differences between the two areas, intensity of production, extent of environmental pollution, or asbestosis) explains satisfactorily the marked apparent difference in cancer risk from amosite and crocidolite in the Transvaal compared with crocidolite in the north-western Cape. The apparent lack of effect of Transvaal crocidolite led Webster⁴ and Wright⁵ to doubt that crocidolite is the important causal agent of mesothelioma in the north-western Cape.

Some authorities have suggested that amosite has not been mined or produced long enough in South Africa for mesotheliomas to develop^{2,6,7}. Examination of available evidence does not support this contention^{8,9}. While it is true that the mining of amosite in the Transvaal is more recent than of crocidolite in the north-western Cape, the interval since amosite was first produced in appreciable amounts is clearly long enough for mesotheliomas to develop (Table 1)^{10,11}, the induction period of the tumour being about 16-55 yr^{1,12}.

The figures for the production (Table 2) show that considerably more amosite than crocidolite has been produced in the Transvaal and that this excess production of amosite over crocidolite was still a feature until about the mid 1950s even when the output of crocidolite for the north-western Cape fields is included. Production figures earlier than 1928 are scanty.

It has been suggested that the population exposed to asbestos in the Transvaal has been small, isolated, and generally not adequately supervised or followed up. This did not seem to us a likely explanation as the medical services in the north-western Cape and the Transvaal do not differ greatly. Moreover, the regulations require the reporting of all deaths in miners and ex-miners in both areas to the Miners' Medical Bureau, Johannesburg. In 1959, two of us visited the four mission hospitals in the Transvaal area to which most of the cases of chest disease in the African population in this region are referred, and the pathologist at Pietersburg to whom specimens are sent. We discussed the problem with medical staff of these hospitals; they were aware of the complications of asbestos exposure and offered to collaborate in the studies. A subsequent visit to the hospitals in 1969 confirmed that in the intervening 10 yr, although numerous patients with asbestosis and some other cases of pneumoconiosis had been seen, only two pleural mesotheliomas had been diagnosed, and one of these had been exposed elsewhere.

We therefore think it is highly likely that there has, in the past 10-20 yr, been a large difference in the risk of mesotheliomas in those working in the two areas in which the closely related amphiboles are mined and milled.

Evidence from intra-pleural inoculations in rat shows that production of mesotheliomas is slightly greater with Cape crocidolite than amosite—an incidence of 59% versus 40% and 68% versus 31% in two large studies^{14,15}. This suggests that amosite can produce tumours when present in the pleural cavity, though possibly slightly less readily than crocidolite and after a longer interval.

Table 1 Approximate Dates of Discovery and Development of Asbestos in South Africa^{10,11}

Type	Discovery	Start of active production	Period between start of active production and present time (yr)
Amosite	1907	1914-1917	51
Crocidolite (north-eastern Transvaal)	1905	1920	48
Crocidolite (north-western Cape)	1803-1806	1893	75

Table 2 Production of Asbestos (Thousands of Tons) from the North-western Cape and North-eastern Transvaal Areas over the Last 40 yr¹³

Years	North-eastern Transvaal Amosite	North-eastern Transvaal Crocidolite	Ratio of amosite produced to crocidolite produced (north-eastern Transvaal)	North-western Cape Crocidolite	Ratio of amosite produced to crocidolite produced (north-western Cape)
1928-1935	35	0.1	350	31	1.1
1936-1943	118	13	9.1	49	2.4
1944-1951	237	48	4.9	85	2.8
1952-1959	446	126	3.5	345	1.3
1960-1968	639	83	7.7	806	0.8

Official production figures provided by *Quarterly Reports on Industrial Minerals*, Union of South Africa Department of Mines (up to 1960) and by *Quarterly Reports on Minerals*, Republic of South Africa (from 1961 onwards).

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Possible Biological Importance of Fibre Diameters of South African Amphiboles

In the preceding communication, Harington, Gilson and Wagner¹ comment on the need to explain the big difference in the number of mesotheliomas reported from the crocidolite mining area of the north-western Cape compared with that in the Transvaal where both crocidolite and amosite are mined. Although the experimental results they quote can be accepted as evidence on the relative carcinogenicity of the various types of asbestos, the intra-pleural inoculation method used to introduce these materials does not take account of the natural factors involved when the fibres are inhaled.

It has been shown that the diameters of fibres have an important influence on the respirability of these particles², so we have looked at the fibre diameter distribution of crocidolite from several mines in the north-western Cape and of crocidolite and amosite from mines in the Transvaal. The fibres for measurement by electron microscopy were produced by standard treatment in pestle and mortar from rock samples or commercial asbestos. Fig. 1 shows the marked difference in the minerals from the two areas. The fibre diameter distributions for the asbestos samples from the north-western Cape fall into one band well separated from those from the Transvaal which are also grouped together. These results confirm earlier observations that an amphibole asbestos comminuted by any one of a variety of different milling methods produces a characteristic diameter distribution not closely related to the method of preparation³. It is specially interesting that the diameter distributions of the crocidolite and amosite from the Transvaal

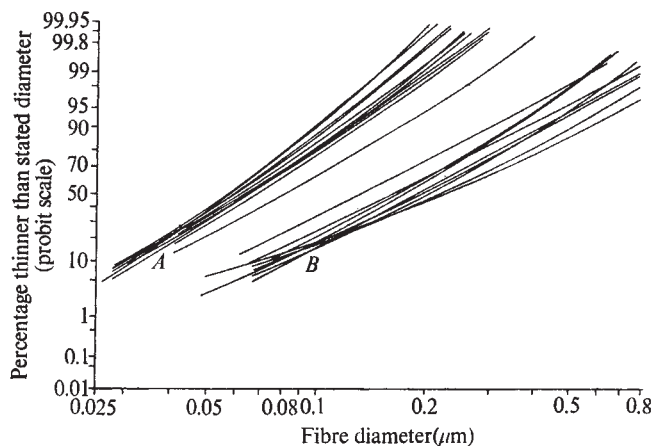


Fig. 1 Measurements of fibre diameters of asbestos from eleven crocidolite mines in the north-western Cape and from different levels in two crocidolite and three amosite mines in the Transvaal. A, North-western Cape (crocidolite); B, Transvaal (amosite, crocidolite).

lie within the same band: one specimen from Malips Drift with abutting crocidolite and amosite layers showed almost identical diameter distributions for the two types of fibres.

The mean fibre diameters for the samples were: north-western Cape crocidolite 0.073 μm , Transvaal crocidolite 0.212 μm , Transvaal amosite 0.243 μm . Thus on the basis of diameter distributions of fibres, the Transvaal crocidolite and amosite are classified together and differ by a factor of about three from the finer crocidolite fibre from the north-western Cape. Crocidolite from the Transvaal and north-western Cape cannot therefore be considered as identical as claimed by Wright⁴.

Electron and optical microscopy show that in the comminuted minerals from the north-western Cape and the Transvaal the fibre length is on average proportional to diameter. Transvaal fibre is therefore on average about thirty times the volume (mass) of that from the north-western Cape. It follows that for a given mass of material comminuted, north-western Cape crocidolite produces about thirty times as many fibres as crocidolite or amosite from the Transvaal.

There is another way in which this difference in fibre size will produce higher concentrations of airborne dust in the north-western Cape than in the Transvaal. For a fibre of high ratio of length to diameter (virtually all the north-western Cape and Transvaal fibres are in this category) the free falling speed (a measure of particle aerodynamic size) is nearly proportional to the diameter squared and increases only slowly with increase in length². Applying this to the results in Fig. 1 we calculate that on average the free falling speed of Transvaal fibre is about nine times that of fibre in the north-western Cape. The Transvaal fibres are therefore less likely to become airborne and will settle more rapidly during production or from the tailings dumps. If we take account of the greater length as well as the greater diameter of the Transvaal fibre, there is a further small increase in these differences between the dusts in the two areas.

The differences in the diameter and length of the fibres are also likely to have an important effect on the ease of penetration to the periphery of the lung and the pleura where the tumours develop. We calculate that the aerodynamic diameters of the north-western Cape fibres have a range from about 0.09–0.8 μm compared with 0.2–2.5 μm for the Transvaal fibres. Because of their greater aerodynamic size, a higher proportion by mass of Transvaal fibres will be deposited in the larger airways by gravitational settling and inertial impaction. Particularly because of interception in the finer airways Transvaal fibre will reach the periphery of the lung much less efficiently than the shorter north-western Cape fibres.

The dose of dust to the larger airways may thus be sufficient to produce asbestosis in both geographical areas. This accords with findings by Sluis-Cremer⁵. But there could be a marked difference between the fibres in the two areas if penetration to the deeper alveoli adjacent to the pleura is an important factor in the production of mesotheliomas.

We thank Mr L. N. Kuyper and Mr H. B. Dugmore for arranging to supply the samples.

Note added in proof. In recent months we have been able to obtain confirmation of these striking differences between north-western Cape and Transvaal fibres from examination of air samples collected in the two areas.

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Effect of Concanavalin A on Lymphocyte-mediated Cytotoxicity

CELLULAR immunity is of primary importance in graft reactions. Sensitization seems to be based on surface interactions between small lymphocytes and cell-bound transplantation antigens; the effector mechanism involves contact interactions between sensitized lymphocytes and the grafted cells. Lymphocytes recognize cell membrane antigens and cause injury to the grafted cells essentially by cell surface processes, the nature of which is not clear. The application of surface-active substances, at both the sensitization and the effector phase, could be expected to make clear some aspects of cell contact interactions involved in graft reaction. We have studied, therefore, the effects of concanavalin A (conA) on a graft reaction system. ConA is a protein molecule with two sugar-binding sites¹⁻⁶, which can interact with sugar moieties on cell surfaces⁷⁻⁹. The graft reaction system we used^{10,11} involved the sensitization of rat lymphocytes against mouse antigens by culturing the lymphocytes on monolayers of mouse fibroblasts for 5 days. This sensitization is characterized by a morphological transformation of small lymphocytes into medium or large cells¹². The sensitized lymphocytes are capable of lysing mouse cells which are antigenically identical to the sensitizing cells. Antibodies are not produced, and the lytic reaction thus requires direct contact between the lymphocytes and the target cells¹³. The rate of lysis is measured by plating the sensitized lymphocytes on fibroblasts labelled with $\text{Na}^{51}\text{CrO}_4$ and measuring the amount of radioactivity released into the culture medium^{14,15}.

The effect of conA (Miles Yeda, Rehovot; crystallized three times) was measured on both the sensitization and the effector (lytic) phase of the *in vitro* graft reaction system described.

Sensitizing monolayers were prepared by seeding 3×10^6 fibroblasts per 60 mm plastic dish: Test monolayers were prepared by seeding 0.75×10^6 cells per 35 mm dish. The fibroblasts were either from mouse (C3H or C57Bl) or rat (Lewis or BN) embryos. All monolayers were exposed to 2,000 r. of X-irradiation 1-2 days after plating. Test mono-

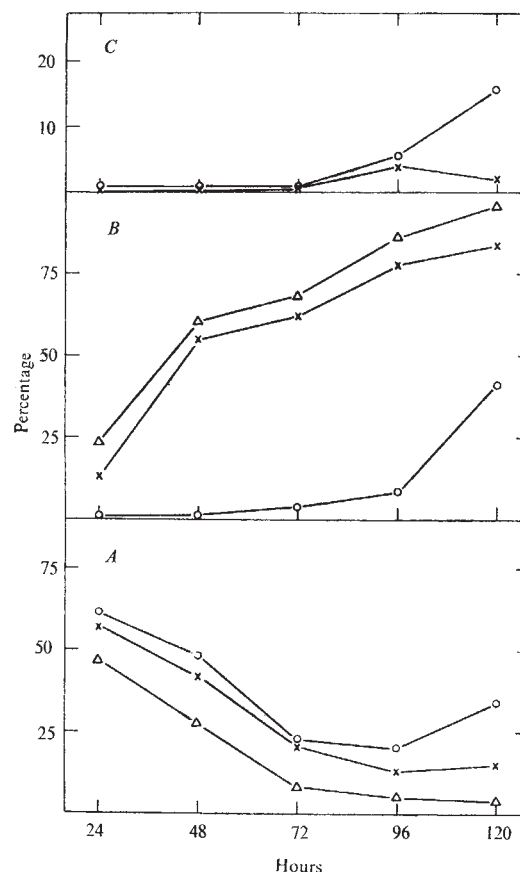


Fig. 1 Changes in cell number, size and replication rate of sensitized lymphocytes are shown as a function of time: *A*, percentage of surviving lymphocytes; *B*, percentage of transformed lymphocytes; *C*, percentage of lymphocytes in division. Lewis lymphocytes were cultured on: ○, C3H monolayer; ×, C3H monolayer plus $50 \mu\text{g ml}^{-1}$ of conA; Δ, $50 \mu\text{g ml}^{-1}$ conA in the absence of fibroblast monolayer. Lymphocytes were collected 1, 2, 3, 4 and 5 days after plating, and were counted differentially with respect to size (small cells distinguished from medium and large cells). To determine rate of replication, colchimid at a final concentration of 10^{-5} M was added to cultures. Cells were collected 3 h later, fixed and stained with aceto-orcein using the method of Hirschhorn¹⁷. One thousand lymphocytes were counted. The percentage of lymphocytes in division represents the number of cells at the metaphase stage, per total number of cells counted.

layers were incubated overnight with $1.5 \mu\text{Ci}$ of $\text{Na}^{51}\text{CrO}_4$ (Radiochemical Centre, Amersham). Lymphocytes from Lewis rat lymph nodes were seeded, 30×10^6 cells per 60 mm dish or $3-4 \times 10^6$ per 35 mm dish. The culture conditions and the experimental procedures have been described^{15,16}. Lymphocyte-mediated cytotoxicity was expressed as the percentage of ^{51}Cr released into the medium to the total ^{51}Cr of the culture. All measurements were made in triplicate. In each experiment the spontaneous release of ^{51}Cr from intact monolayers was determined in parallel, and the values were subtracted from the experimental result. The deviation from the mean of the experimental values did not exceed $\pm 8\%$.

We tested the effect of conA on survival, transformation and replication of lymphocytes cultured either with or without mouse fibroblasts. The results (Fig. 1) show that during the first 3 days in culture, the lymphocyte population in all three experimental groups (fibroblasts, fibroblasts plus conA and conA without fibroblasts) decreased in number (Fig. 1*A*). Lymphocytes cultured on fibroblasts showed a tendency at day 4-5 to increase in population size (Fig. 1*A*), increase in rate of cell replication (Fig. 1*C*) and increase in percentage of large cells (Fig. 1*B*). In the groups exposed to conA, the rate of cell replication was reduced (Fig. 1*C*), and the percentage of

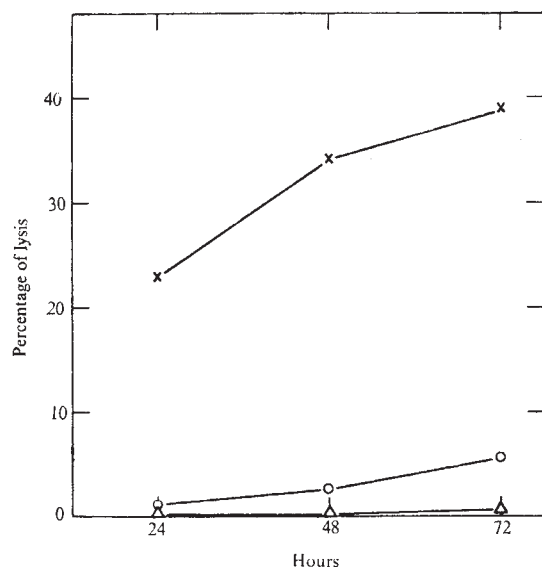


Fig. 2 The effect of conA on the rate of lysis is shown in: x, cultures with $50 \mu\text{g ml}^{-1}$ of conA; O, cultures with no conA; Δ , spontaneous release in the presence of $50 \mu\text{g ml}^{-1}$ conA. Lymphocytes (4×10^6) were plated directly on C3H test monolayers. The rate of lysis was measured after 24, 48 and 72 h of incubation.

large cells increased considerably compared with untreated lymphocytes. In the presence of conA, small lymphocytes were all transformed to medium size within 24 h, and after 48 h more than 50% were large lymphocytes.

To test the effect of conA on the adherence of the lymphocytes to the fibroblast monolayer, cultures were incubated for 24 h in the presence of $50 \mu\text{g ml}^{-1}$ conA. The monolayers were then washed by repeated pipetting and the culture medium was discarded. The monolayers were treated with trypsin and samples of the trypsinized monolayers were examined microscopically. ConA-treated cultures showed a five-fold increase in the number of lymphocytes recovered from the fibroblasts, compared with cultures not treated with the drug. These results support the idea that conA does provide a molecular bridge between the two cell types.

Because conA triggers blastogenesis and causes adherence of lymphocytes to the fibroblasts, experiments were performed

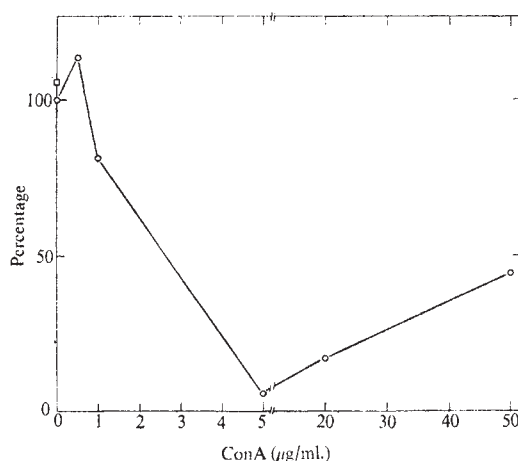


Fig. 3 The effect of conA applied during sensitization on the lytic capacity of the effector lymphocytes shown with: O, conA applied only to the sensitizing monolayer; $\square$, conA ($50 \mu\text{g ml}^{-1}$) applied only to the test monolayer. Lymphocytes were cultured in the presence of from 0 to $50 \mu\text{g ml}^{-1}$ of conA. On day 5 the lymphocytes were collected and 3×10^6 cells were plated on each test monolayer. The rate of lysis was measured 46 h later. Here 100% equals 39% of net lysis.

to test whether the drug-treated lymphocytes possess lytic capacity. First (Fig. 2) lymphocytes were plated directly on labelled fibroblasts in the presence of conA, so that cell-mediated lysis could be measured from the onset of lymphocyte-fibroblast interaction. The results indicated that when lysis was measured 24, 48 and 72 h after plating, conA-treated lymphocytes had a significantly higher lytic capacity than the untreated lymphocytes. The high rate of lysis measured at 24 h after plating was impressive when compared with the untreated cultures which did not cause any measurable lysis. These results indicate that conA confers on lymphocytes cytolytic activity while interacting with fibroblasts. This activity is not dependent on replication of the lymphocytes, because during this period (first 3 days of culture) no lymphocyte replication was detected (Fig. 1C). ConA-mediated lysis does not require complete transformation to large cells, because lysis was measured after 24 h, at which time the lymphocytes were medium sized.

In the second experiment (Fig. 3) lymphocytes were cultured for 5 days on C3H monolayers, either with conA at different concentrations or without conA. On the fifth day the lymphocytes were collected, washed and transferred to labelled test monolayers. ConA was found to affect the lytic capacity of the treated lymphocytes. With conA at a concentration of $0.5 \mu\text{g ml}^{-1}$ the lytic activity was slightly increased, although at higher concentrations lysis decreased, with respect to lytic activity obtained by antigenically sensitized lymphocytes (sensitized in the absence of conA). The effect cannot be attributed to conA carried over to the test cultures, because lymphocytes sensitized for 5 days on C3H fibroblasts in the absence of conA, when tested in the presence of conA, did not have any inhibition of their lytic activity (Fig. 3). The results (Figs. 2, 3) show that the lytic capacity of conA-treated lymphocytes is lost after a long period of treatment. They also suggest that conA interferes with the process of sensitization induced by the monolayer.

Does the lytic capacity, conferred on lymphocytes interacting with fibroblasts in the presence of conA, possess antigenic specificity determined by the sensitizing fibroblasts? To test this, Lewis lymphocytes were cultured on C3H or Lewis fibroblasts in the presence of conA. Another group of lymphocytes was cultured with conA in the absence of fibroblasts. After 48 h the lymphocytes of each group were tested for lytic activity on C3H and Lewis test monolayers, in the presence of $50 \mu\text{g ml}^{-1}$ conA. No antigenic specificity was found. Lymphocytes cultured on either C3H or Lewis fibroblast monolayers produced the same level of lysis when tested on C3H or Lewis test monolayer (Table 1). Lymphocytes cultured with conA only, in the absence of fibroblasts, had the same lytic capacity as lymphocytes cultured with the drug in the presence of a monolayer. The lack of immunospecificity in the presence of conA is further demonstrated in the experiment summarized in Fig. 4. Lymphocytes, cultured for 1, 2, 3 and 4 days with or without fibroblast monolayers, and in the presence or absence of conA, were tested for lytic activity. In this experiment

Table 1 Tests for Specific Lysis in the Presence of ConA

Sensitizing monolayer	Test monolayer	% lysis
C3H	C3H	24.3
	Lewis	26.4
Lewis	C3H	19.1
	Lewis	21.5
No monolayer	C3H	32.0
	Lewis	24.7

Lewis lymphocytes were cultured either on C3H or Lewis monolayers or with no monolayer. After 48 h the lymphocytes were collected and 4×10^6 lymphocytes were transferred to either C3H or Lewis test monolayers. Concentration of $50 \mu\text{g ml}^{-1}$ of conA was maintained throughout the experiment. The rate of lysis was measured 40 h later.

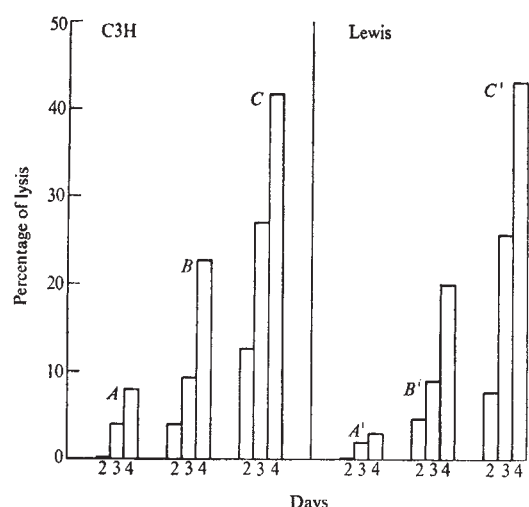


Fig. 4 Tests of specific lysis of lymphocytes sensitized either by conA, fibroblast monolayer or both; Lewis lymphocytes were cultured on: A and A', C3H fibroblast monolayer; B and B', C3H fibroblast monolayer with the addition of $50 \mu\text{g ml}^{-1}$ conA; C and C', with no monolayer but with conA. The lymphocytes were collected after 2, 3 or 4 days and were plated on either C3H or Lewis test monolayers; 4×10^6 cells were plated in each culture. The rate of lysis was measured 24 h later.

(Fig. 4) no conA was added to the test cultures. Our experiments with labelled conA indicate, however, that some remained bound to the lymphocytes.

Thus, a population of lymphocytes has blastogenic and lytic capacity after conA treatment, as does a population of cells sensitized by fibroblasts. Because the cell extract and media of conA-treated and antigenically sensitized cultures do not have any measurable lytic activity, it seems that lysis in both systems is cell-mediated (refs. 11, 13 and unpublished results). The difference between the two treatments is that the lytic processes activated by conA have no antigenic specificity, whereas rat lymphocytes sensitized on mouse fibroblasts have a significant antigenically specific lysis^{11,13}. Analysis of the effector phase of immune lysis revealed that it comprised two distinct processes: the activation, by a specific antigen, of the effector lytic mechanism, and the lytic process itself, which is antigenically non-specific¹⁸. Therefore, conA may replace the specific antigen by triggering the lytic reaction of unsensitized lymphocytes.

Evidence has accumulated which indicates that the lytic potential of the lymphocyte population is an inherent property of these cells, rather than being given to them by an antigenic stimulation. We have demonstrated that the lytic capacity of conA-treated lymphocytes is expressed by cells which neither underwent replication nor completed morphological transformation. We also found that the inhibition of DNA synthesis does not abolish the lytic capacity of these lymphocytes (article in preparation). In antigenically sensitized lymphocytes it has been shown that there is no measurable synthesis of new species of protein or RNA¹⁹.

Blast transformation and cytotoxicity are separate expressions of lymphocyte stimulation, as Perlmann *et al.*²⁰ inferred from experiments on the effect of conA on human lymphocytes. They found that conA-treated human lymphocytes undergo transformation without developing cytotoxicity to chicken erythrocytes. Furthermore, such lymphocytes are incapable of affecting phytohaemagglutinin-induced cytotoxicity.

The conA-induced lysis in our system might provide a model for the lysis of cells activated by specific antigens. The sensitization phase in cell-mediated immunity might be based on the selection, from the normal lymphocytic population, of cells with both the capacity to interact with specific antigens and

the potential for lysis. The percentage of these cells increased by replication, so that on day 5 the population became rich in antigenically specific effector cells. The actual activation of the lytic mechanism by antigens, and the lytic process itself, might be similar, if not identical, to the activation of non-specific lysis by conA. Whether the actual injury to fibroblasts depends on the adhesion of lymphocytes to the fibroblasts through a "bridge" formed by the conA molecule is still under investigation. An alternative mechanism is that a conA-treated lymphocyte might acquire the capacity to mediate cytotoxicity, independent of the presence of conA during lysis. According to our preliminary work, the first mechanism seems more likely.

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Long Term Effect of Vasopressin on the Maintenance of a Conditioned Avoidance Response in Rats

RATS subjected to posterior lobectomy are unable to maintain a shuttle-box avoidance response without reinforcement¹, but normal behaviour can be restored to that of sham-operated controls by treatment with pitressin tannate in oil, a relatively crude extract of posterior and intermediate pituitary lobes. Long acting preparations of ACTH and α -MSH can also restore the normal rate of extinction of an avoidance response in posterior lobectomized rats². Pitressin as well as ACTH analogues, including α -MSH, delay the extinction of an avoidance response also in intact rats^{3,4}. Treatment with pitressin tannate in oil, however, preserves a conditioned

Table 1 Effect of a Single Injection of an Analogue of ACTH or Lysine Vasopressin on the Rate of Extinction of a Pole Jumping Avoidance Response

Treatment	First extinction session	Number of conditioned avoidance responses (ten trials)			
		Subsequent extinction sessions (h after injection)			
		2	4	24	72
Saline 0.5 ml. subcutaneously	9±0.5	5±0.6	2±0.6	0±0.0	0±0.0 (4)
ACTH 4-10 100 µg subcutaneously	9±0.4	8±0.0	7±0.3	1±0.6	0±0.0 (5)
Lysine vasopressin 1 µg subcutaneously	9±0.3	9±0.2	9±0.2	9±0.0	9±0.0 (5)

Figures are means ±s.e. The number of animals is given in brackets.

The synthetic lysine vasopressin had a potency of 60 IU/mg. ACTH 4-10⁴.

Male white rats weighing between 120 and 140 g were conditioned to jump onto a pole⁵. The conditioned stimulus (CS) was a light presented for 5 s. Rats which failed to jump onto the pole within 5 s after CS onset received unscrambled electric shock (US) applied through the grid floor. A 540 V a.c. shock source was used with a current limiting resistor in series with the animal; the short circuit value of the shock was 0.2 mA. The CS and US were terminated at the moment of the response. Ten conditioning trials were given each day for 3 days with an intertrial onset interval averaging 60 s. Rats which made ten or more avoidance responses during the thirty conditioning trials were used for extinction studies which were started on the fourth day of the experiment.

Extinction sessions of ten trials each were run on the same schedule as that used during acquisition except that the unconditioned stimulus of shock was not applied if the rat did not make a response. The maximum CS duration was 5 s. Only rats which made eight or more avoidances in the first extinction session were used for subsequent extinction sessions. Animals were injected subcutaneously with the respective peptides immediately after the last trials of the first extinction session. Subsequent extinction sessions were run 2, 4, 24, 48 and 72 h later.

Table 2 Effect of a Single Injection of Various Peptides on the Rate of Extinction of a Pole Jumping Avoidance Response

Treatment	First extinction session	Number of conditioned avoidance responses (ten trials)		
		Subsequent extinction sessions (h after injection)		
		2	4	24
Saline 0.5 ml. subcutaneously	9±0.5	4±0.7	1±0.5	0±0.0 (4)
Oxytocin 1 µg subcutaneously	9±0.3	3±1.1	1±0.5	0±0.0 (5)
Angiotensin II 1 µg subcutaneously	9±0.4	3±1.1	2±0.8	0±0.4 (5)
Saline 0.5 ml. subcutaneously	8±0.3	3±0.3	0±0.4	0±0.0 (6)
Insulin 1 µg subcutaneously	8±0.3	4±0.5	1±0.4	0±0.2 (7)
Growth hormone 1 µg subcutaneously	9±0.3	4±0.3	1±0.4	0±0.2 (7)

Figures are means ±s.e. The number of animals is given in brackets.

The synthetic oxytocin ('Piton-S') had a potency of 60 IU/mg; synthetic angiotensin II was 'Hypertensin' (Ciba); purified bovine insulin had a potency of 25.6 IU/mg; purified growth hormone had a potency of 1.9 IU/mg.

Table 3 Effect of a Single Injection of an ACTH-Analogue or Lysine Vasopressin Immediately, 1 and 6 h after the First Extinction Session on the Rate of Extinction of a Pole Jumping Avoidance Response

Treatment and time of injection after the first extinction session	First extinction session	No of conditioned avoidance responses (ten trials)	
		Subsequent extinction sessions (h after injection)	
		24	48
Saline 0.5 ml. subcutaneously	9±0.4	3±0.4	0±0.3 (6)
ACTH 4-10 100 µg subcutaneously	9±0.4	4±0.3	1±0.5 (6)
Lysine vasopressin 1 µg subcutaneously	9±0.3	9±0.3	9±0.3 (7)
1 hour			
Saline 0.5 ml. subcutaneously	9±0.3	2±0.5	0±0.0 (5)
Lysine vasopressin 1 µg subcutaneously	9±0.4	7±0.4	6±0.4 (5)
6 hours			
Saline 0.5 ml. subcutaneously	9±0.3	2±0.4	0±0.0 (5)
Lysine vasopressin 1 µg subcutaneously	9±0.3	4±0.3	1±0.4 (5)

Figures are means ±s.e. The number of animals is given in brackets.

avoidance response whether the treatment is given during acquisition or extinction, while ACTH or ACTH analogues inhibit extinction most markedly during the treatment period³. These observations suggest that the mechanisms by which pitressin and analogues of ACTH affect conditioned avoidance behaviour are basically different. The fact that rats treated with pitressin retain the avoidance response for a considerable time after treatment suggests that this preparation contains peptides

which interfere with long term memory storage, but that peptides with a configuration like that of ACTH participate in short term (trial to trial) memory processes³.

The principle in the posterior pituitary responsible for this long term effect on maintenance of an avoidance response during extinction has not been identified; appropriate experiments have so far been difficult to perform because the preparation of long acting vasopressin or oxytocin is not feasible.

My experiments circumvent this difficulty. From an experiment in which the influence of various peptides not prepared for long lasting activity on the retention of a pole jumping avoidance response were studied, I conclude that vasopressin is the peptide in the pitressin preparation responsible for the long term effect on the maintenance of an avoidance response.

In the first experiment the influence of an analogue of ACTH (ACTH 4-10) and of lysine vasopressin was studied. ACTH 4-10 (100 µg) significantly inhibited extinction of the avoidance response 2 or 4 h after injection compared with saline treatment (Table 1). The effect of this peptide had disappeared 24 h after its administration. In contrast, 1 µg of lysine vasopressin not only inhibited extinction of the avoidance response at 2 and 4 h but also 24 and 72 h after injection.

To determine the specificity of vasopressin in this respect, other structurally and physiologically related peptides were tested in the same situation. Neither oxytocin nor angiotensin II in a dose of 1 µg affected the maintenance of the avoidance response (Table 2). This was also true for similar amounts of insulin and growth hormone.

Finally, I attempted to determine if there was a critical period for the effect of lysine vasopressin on the maintenance of the avoidance response. For this purpose, rats were injected immediately, or 1 or 6 h after the last trial of the first extinction session. Extinction sessions were run 24 and 48 h after the first extinction session. For comparison the effect of ACTH 4-10 was also measured in the experiment in which the peptides were administered immediately after the first extinction session.

As in the first experiment ACTH 4-10 did not affect the rate of extinction of the avoidance response when measured 24 and 48 h later, whereas lysine vasopressin injected immediately after the first extinction session again resulted in complete inhibition of extinction (Table 3). Administration of lysine vasopressin 1 h after the first extinction session also inhibited the rate of extinction, although the effect did not seem to be as complete as that produced by the immediate injection. When administration was delayed for 6 h, the effect on extinction was not observed. Accordingly, there seems to be a critical period for the influence of vasopressin on the maintenance of the avoidance response; to be completely effective vasopressin must be administered within 1 h after the extinction session.

Although the number of animals in the individual experiments was small, the reliability of the results is compelling. These experiments confirm previous studies with a relatively crude extract of the posterior pituitary, pitressin and the analogue ACTH, α -MSH, administered as long acting preparations on the maintenance of a shuttle-box avoidance response³. In these experiments, using a different situation from that referred to, it has been demonstrated that the peptide in the pitressin preparation responsible for the long term effect on extinction of the avoidance response is vasopressin. The effect is highly specific, for the structurally closely related peptide oxytocin, at least in the amounts used, is without effect. Angiotensin II which is an even more powerful pressor substance than vasopressin also has no effect on extinction, which indicates that circulatory changes are not involved. It seems that this is also true with respect to the influence of vasopressin on carbohydrate metabolism⁶ because neither insulin nor growth can mimic the behavioural effect of vasopressin.

In contrast to the immediate and relatively brief effects of ACTH 4-10, lysine vasopressin facilitates consolidation of a conditioned avoidance response. The way in which these peptides act is as yet unknown. These findings suggest, however, that consolidation of learned responses may depend upon the presence of certain neurogenic peptides.

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Secretory Cells in the Premature Human Lung Lobule

IN this report, we describe cells with the ultrastructural characteristics of neurosecretory cells in the lung lobules of prematurely born human infants. The existence of these cells in the lungs of newborn infants was first suggested by Lauweryns and Peuskens on the basis of light and fluorescence microscopy¹. Previously, analogous cells had been found in the lungs of infants² and in adult lung³ with the light and electron microscopes^{4,5}. There is no certainty that all authors are reporting the same cells, even within their own material; for example, Feyrter proposes a change in their argyrophilic properties during the first few years of life⁶. The functional significance of these cells in premature infants includes the possibility that they modulate lobular secretion during foetal life, which would thus regulate lobular growth according to Towers's theory⁷; and also that they may be one of the still unknown factors which modulates normal perinatal cardiopulmonary adaptation⁸ and which plays a role in respiratory distress syndrome, a condition with clinical⁹ and anatomical¹⁰ characteristics of hypoperfusion.

Lungs were obtained from two infants weighing 600 and 1,600 g as soon as possible after death and dissected under the microscope. Material was fixed in phosphate buffered glutaraldehyde (pH 7.2), post-osmified, embedded in 'Epon', sectioned, and stained by Reynolds's method¹¹. The characteristic cells occur intercalated among mucosal cells of small bronchioles and also among respiratory epithelia (of the intersaccular septae¹²). The relative frequency of cells and the number of granules per cell profile was greater in the smaller infant. The typical cell (Fig. 1) is small, irregularly cuboidal, and extends below the usual level of other cells lining the lung nearby but its profile does not usually touch the lumen. Typically, it does not insinuate long narrow protoplasmic processes between or around cells. The basal lamina is usually similar to that of neighbouring cells. The secretory cells are not intimately associated with smooth muscle. A capillary is usually quite close.

The numerous inclusions (Fig. 1) have a centrally located core of tightly packed material, surrounded by a clear rim which is occasionally traversed by amorphous material sometimes in a radial pattern. Surrounding the whole is a typical unit membrane. Characteristically, the core measures about 80 × 100 nm, the envelope 110 × 130 nm, and the shape is ovoid. In the 1,600 g infant, the granules were less dense and more varied in size.

The cytoplasm is typically quite dense at 600 g, and less so at 1,600 g. There is a moderate amount of endoplasmic reticulum. The rough type occurs as linear or broadly curved lamellae, the cisternae of which sometimes contain fine amorphous material. The smooth type is sometimes vesicular. The Golgi zone, which is not prominent, is not associated closely with the granules. Multivesicular bodies are uncommon and lamellated inclusions as seen in alveolar type II cells were not noted. Residual (lipofuscin) bodies were not seen. Extrusion of granules across the cell membrane was not seen.

These findings suggest a relationship between the fluorescent granulated kinin-like, amine-like, or neurosecretory cells

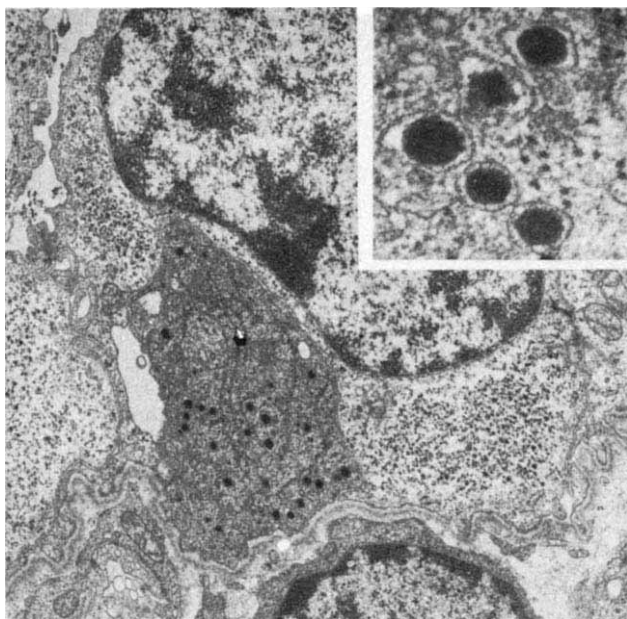


Fig. 1 Electron micrograph of a characteristic granulated cell with its quite dense cytoplasm and numerous inclusions intercalated among the other bronchiolar mucosal cells and close to the basement membrane ($\times 12,000$). Inset: higher magnification of the typical dense-cored vesicles ($\times 28,500$).

previously observed in this laboratory and those described here. They also show some classic resemblance to other so called APUD cells¹³ that are believed to form a family to which cells of the carotid body, adrenal medulla, intestinal chromaffin tissue and tissues are related. The materials isolated from these cells in other laboratories may be broadly characterized as vaso-active and musculo-active, and some are mediators of neuronal activity. Thus, the location of these cells in the bronchiolar or apical portion of the normal foetal lobule is consistent with the idea that this locus is probably a regulatory centre of lobular activity⁷ and the observation that muscular bronchioles and vessels, nerves and lymphatics are closely grouped in this vital area even in the 600 g infant¹⁴.

The cells described are clearly different from those described by Gmelich *et al.*¹⁵ with respect to cell profile, density of cytoplasm, and appearance of organelles such as endoplasmic reticulum and lipid inclusions. Furthermore, some of our cells occur in intersaccular septae, a location not previously known for Kultschitsky cells. At this peripheral site, they are well located for possible interaction with the pulmonary microcirculation, including venules and lymphatics. Perhaps the foetal lung, which subserves secretion rather than ventilation and which differs radically in morphology¹⁴ as well as function⁷ from the adult, requires a correspondingly different arrangement and variation of its family of APUD cells. In the case of adrenaline and noradrenaline, Coupland showed that glutaraldehyde-osmium procedures at pH 5.8 effectively differentiated one type of granule from another¹⁶. Although we do not know what is in the granules described here, and although our technique required a higher pH, it is interesting that on average the granules become less electron opaque in the more mature infant. An analogous change in staining reaction occurs in differentiating adrenal chromaffin tissue¹⁶.

We cannot avoid associating the presence of these cells with a possible regulatory activity in the foetal and neonatal lobule. If these postulated activities are aspects of normal foetal lobular function, then they might continue to mediate lobular ventilation and perfusion in prematurely delivered infants. Thus, disturbed postnatal function or lesions of these cells might disrupt the complex integration of the dynamic lobule. On the other hand, normal postnatal function of these

cells, if directed towards integrative foetal patterns of the secretory lung might be inappropriate for a ventilated lung. This might be especially important for lung diseases in premature infants.

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The Spindle at Metaphase

OSTERGREN¹ suggested that, before and during metaphase, the mitotic and meiotic chromosomes are subjected to pole-directed forces α , which orient the chromosomes at the equator of the spindle and separate them at anaphase. He also postulated a centrifugal force, γ , moving the chromosomes to the periphery of the metaphase plate; but he could suggest no physical explanation for γ . McIntosh *et al.*² developed Ostergren's hypothesis, but failed to account for several important features of mitotic and meiotic spindles, including γ .

I suggest that the spindle and chromosomes are not subjected to any tension in the chromosomal or continuous fibres before and during metaphase, but that enough pressure is exerted along the fibres to orient the chromosomes and to generate Ostergren's force γ . The chromosomes move apart autonomously at metaphase³⁻⁵, after which further separation is organized by the kinetochores and their associated fibres.

In support of this hypothesis, the following observations on dividing *Schistocerca gregaria* testis cells are relevant: (1) The shape of the spindle is that of two cones placed base to base (Fig. 1). (2) The angle θ at the base of the cones is acute. In the mitotic spindle at metaphase, $\theta = 37^\circ$, in both meiotic spindles $\theta = 54^\circ$. (3) The chromosomes at metaphase are arranged in a circle around the periphery of the two cone bases, like a necklace. There are no chromosomes at the centre of the metaphase plate, but there are small vesicles and ribosomes in this area. (4) In my electron micrographs there are very few microtubules running from pole to pole across the metaphase plate. Microtubules running from the kineto-

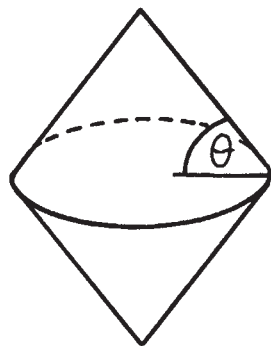


Fig. 1 Double cone structure of spindle.

chores to the poles tend to be associated in bundles of twelve to twenty. (5) At anaphase, it can be seen in the light microscope that the chromosomes part along paths at 2θ to one another. It is important to note the absence of large numbers of close-packed microtubules in the centre of the spindle, or any obvious radial structure, any structure joining the chromosomes one to another as they lie in a circle, or any structure binding the chromosomes to cell membrane or to any microtubules lying outside the metaphase plate.

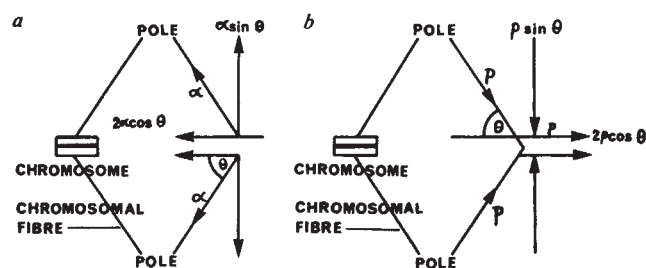


Fig. 2 Schematic diagram of the spindle. *a*, The prometaphase-metaphase situation envisaged by Ostergren. *b*, The situation when the force acting along the fibres is reversed.

Fig. 2 (*a*) illustrates the prometaphase-metaphase situation as envisaged by Ostergren. Suppose the force α acts along the chromosomal fibre, or microtubule bundle, towards the pole. This force can be resolved into two components at 90° . The diagram shows that the only net force on the chromosomes is the inward directed force of $2\alpha \cos \theta$. Movement into the centre of the spindle would therefore be expected during metaphase. This has not been recorded. I have not observed anything to oppose this movement; various techniques^{6,7} indicate that the density of the spindle is about the same as the surrounding cytoplasm, and micromanipulation⁸ shows that lateral chromosome movement is not impeded. Izutsu⁹ made a chromosome bridge, and observed that the abnormal bivalent did move into the centre of the spindle at anaphase, showing that there is no physical barrier to this movement. One can therefore be confident that any tension in the chromosomal fibres would produce such a movement unless the sister chromosomes are already separate.

Fig. 2*b* illustrates the situation if the direction of the force (say, force P) acting along the fibres is reversed. The chromosomes are subjected to the balanced forces $P \sin \theta$ tending to hold them in equilibrium at the equator of the spindle, and the force $2P \cos \theta$ which holds them at the periphery of the metaphase plate. $2P \cos \theta$ would correspond to Ostergren's force γ , and $P \sin \theta$ replaces force α in keeping the chromosomes at the equatorial position.

I suggest that the force P along the kinetochore-pole fibres (and the continuous fibres) is generated by the lengthening of

the microtubules, between chromosomes and poles. It has been shown that microtubules lengthen by growth from one end^{10,11}. Microtubules are rigid structures^{12,13} and so are chromosomal fibres^{5,8,14}. If microtubules growing from the kinetochores^{2,15,16} engage with those growing from the poles², and are constrained somewhat at the poles⁸, then one would expect to see a slow migration of the chromosomes to the periphery of the metaphase plate. The spindle as a whole would bulge at the equator as has been described¹⁷; further bulging occurs if the chromosomes are experimentally held at metaphase¹⁸. In cells in which the poles are not well defined or are absent, there is little or no bulging at the equator^{9,19-21}. If the growth of fibres from paired kinetochores is slightly unbalanced, one would expect to see oscillations across the plane of the metaphase plate until the fibres from both kinetochores are in equilibrium (that is, the same length). Such oscillations have been described^{22,23}.

It is clear that at the beginning of metaphase, the chromosomes are still mechanically joined^{9,25}. The autonomous separation of chromosomes at the end of metaphase is well documented^{7,16} but unexplained; it may be a corollary of the process of condensation, for condensing chromosomes do not normally stick to one another. Endomitosis^{23,26} is a process in which separation (but not segregation) of sister chromatids occurs in the absence of any spindle. The separation of acentric chromosome arms⁵, and of chromosome arms in mitoses which have been arrested by treatment with colchicine, has also been reported^{4,27}.

After the initial separation, any tension between the chromosomes and poles will not tend to shift chromosomes to the centre of the spindle, so further movement may be organized by the kinetochores and associated fibres without the inward collapse of the double cone structure described here.

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BOOK REVIEWS

Stapledon in Fashion

Human Ecology. By Sir George Stapledon. Second edition. Edited and introduced by Robert Waller. Pp. xi+252. (Charles Knight: London, January 1971.) £2.25.

It is embarrassing in 1971 to have to review so wise and timely a book as this knowing that when he wrote it a quarter of a century ago Sir George Stapledon became so discouraged about its prospects of being read that he left it as an unfinished manuscript. Only now, after the usual costly time lag, has informed opinion begun to catch up sufficiently for some of the message to be understood by some readers.

In this Stapledon shared the fate of a number of scientists of deep and wide ranging imagination, who were respected within a narrow professional field but ignored on wider issues. Tansley, whom he perhaps most nearly resembled, was luckier partly through being even longer lived, coming into his own in his eighties. But Stapledon never lived to see the great current impact of ideas which he had shaped and championed on the pervasive role of ecology in agriculture, in government and in human affairs generally.

This book consists of a closely reasoned argument, cosmic in scope, relating man's current gropings and backslidings to biological principles and to less ephemeral values than have been in fashion lately. It gives few references and is only lightly buttressed with scientific support, yet the steady penetrating perspective of its view, the discriminating choice and use of words, the restrained but prophetic and convincing vision behind it, and the knowledge of its author's scientific and practical achievements go far to disarm criticism. This might not have been the case, however, but for the new awareness of an immense body of data and fully documented case histories which now make it idle to question whether certain *obiter dicta* are soundly based.

While it is quite respectable for a scientist to use his talents in exploring the lithosphere, the atmosphere, the biosphere or even the technosphere, he is still looked on askance for trying to understand the noosphere—the man-made “thinking layer” (as Teilhard de Chardin called it, which since the end of the Tertiary “has spread over and above the world of plants and animals”). Vastly important as its content is, this book raises an issue of even greater significance to science. When, if ever, are

scientists to be permitted, if not encouraged, to apply their gifts and techniques to systematic survey and analysis of the nature of the noosphere, man's crowning inheritance, of which science itself is a fine flower? How many more Stapledons and Tansleys must be either warned off it if they are timid, or live in frustration if they are courageous enough, before it becomes intellectually acceptable to probe into whether the noosphere is round or flat? Mr Waller and the publishers are to be congratulated, not only on making Stapledon's stimulating argument available, but on providing this awkward case history of an issue which science must sooner or later face. The book would, however, have been better without Mr Waller's appendices, which make no more than an anticlimax, and pursue arguments much more questionable than Stapledon's.

E. M. NICHOLSON

Making Decisions

Making Decisions. By D. V. Lindley. Pp. 195. (Wiley-Interscience: London and New York, March 1971.) £1.95.

In many areas of planning efforts at decision making seem to be characterized by unsystematic, irrational methods and procedures. Boards and committees are influenced by the most persuasive speaker, or by irrelevant or misrepresented data. There is little attempt to reduce problems to their fundamental components nor to proceed logically from these. Possible eventualities are rarely listed in a table, they may also be inconsistent. What this book does—far more than to explain and prove the laws of probability—is to advocate clarity and precision and coherence in decision making. Its lucid text and very careful progression to the simple laws of probability make it an ideal introduction to the subject. There is a wealth of practical and amusing examples (and a refreshing absence of absurdly useless data such as those relating to unlikely fatalities in the Prussian army which used to appear in standard works). There are details of actual uses of the laws in military, factory, and private decision making.

In spite of a final chapter devoted to the vagaries of subjective judgment, there is perhaps an overemphasis on logical procedure—designed no doubt to counterbalance loose and imprecise thinking that prevails wherever ordinary people are left to make decisions. A somewhat mechanical approach can lead to surprising inconsistencies (as with the

famous ass who, finding himself equidistant from two identical piles of hay, was unable to decide which to move toward first and so starved). The book has not allowed for this sort of situation where perhaps common sense must triumph.

Furthermore, although there is mention of other methods of decision making, there appear to be omissions. Cost benefit analysis allows us to consider personal taste, maximization of public and of private utilities (together), risks, interest rates and more. Perhaps because these preferences cannot always be quantified (though they may be ranked or otherwise ordered), Professor Lindley avoids this sort of method.

In spite of this, the book reads so clearly and it is so careful and conscientious in presentation that it is to be recommended as an example of purity in style and expression which lends much to its simple and clear explanations of formulae and expressions. This clarity and rationality will influence the reader and will be useful even in the most mundane situations where judgment is called for, and where all too often the arguments will be found to be based on false assumptions and are further confused with irrelevant information. Professor Lindley would like politicians, for example, to rank their priorities on public expenditure. PAULINE JONES

Information Reviewed

Annual Review of Information Science and Technology, Vol. 5. Edited by Carlos A. Cuadra. Assisted by Ann W. Luke. (American Society for Information Science.) Pp. 468. (Encyclopaedia Britannica: Chicago. Distributed in UK by Wiley: Chichester, January 1971.) £8.29.

THE fifth *Annual Review of Information Science and Technology* covers the literature of 1969, the last year of a decade which was both formative and significant in establishing information as an important factor in maintaining world progress. Actually in the year under review there were no spectacular developments; it was application rather than innovation—as one author puts it, it was “fine-polishing and cost reduction”.

It is worth summarizing some of the main trends which are reported. Thus there is the intake and evaluation in one computerized centre of duplicate magnetic tapes from many sources: profes-

sional societies (chemistry, physics and so on), commercial firms, and even governmental bodies (MEDLARS, INIS). However, the intensified use of such tape stores highlights the serious problems of standardization, incompatibilities of format and indexing vocabularies that are not on speaking terms. From a somewhat passive storage role microfilm is rapidly becoming more active in association with computer technology. Computer Output Microfilm (COM) combines the miniaturization and the handling of records. There are indications of the progressive merging of the publishing industries (with their micro-publishing, bespoke information packages and so on) and some documentation services, since "a totally integrated technology for information transfer" would certainly reduce costs.

Perhaps the three most stimulating chapters, and this lies partly in the nature of the subjects and the critical approach of the authors, are those on information needs and uses (Ben-Ami Lipetz), design and evaluation of information systems (F. W. Lancaster and C. J. Gillespie) and library automation (R. H. Parker). Under evaluation a new term "lexicodynamics" is reported, which expresses "the concept of construction, maintenance, use and change of controlled vocabularies for information retrieval purposes". This is an area which is absorbing a great deal of documentation manpower everywhere. Also there is a useful discussion on the evaluation of published indexes and a basic criterion is suggested—unit cost per useful reference retrieved in relation to the total number examined and the search time needed.

On the mechanization of library processes improvements in both hardware and software have made it possible to work within the traditional bibliographical norms, rather than accepting the earlier machine restrictions. Furthermore, this computerization shows signs of coming of age in that it is now being evaluated objectively rather than emotionally. In the chapter on management information systems S. D. Weiss stresses that "the literature . . . lacks both organization and classification", which some documentalists have long suspected. The explanation given is that this interdisciplinary field consists of a number of disparate subjects.

This volume marks the end of the five-year subsidy from the US National Science Foundation, and it now becomes a straight commercial publication. The *Review* has certainly maintained the good standards it set at the beginning—timeliness, variety and quality of the reviewers, indexes both current and cumulative, though greater depth of indexing would be more helpful.

HERBERT COBLANS

Brain and Hormones

Pituitary, Adrenal and the Brain. Edited by D. de Wied and J. A. W. M. Weijnen. (Progress in Brain Research, Volume 32.) Pp. xv+357. (Elsevier: Amsterdam, London and New York, 1970.) £9.

THIS volume covers the proceedings of an international conference on the pituitary-adrenal axis and the nervous system, held at Vierhouten, the Netherlands, in 1969. Each of the forty-two chapters is followed by worthwhile discussion.

The first part of the book covers the first session of the conference and provides details of the latest research on the effect of the nervous system on pituitary-adrenal activity. Present views of feedback control of adrenal steroids on the hypothalamus are given, and some interesting discussions arose on this topic. I hope people will note Professor G. W. Harris's important comment that too little attention has been paid to protein-binding of adrenal steroids in the blood; possibly measurements of the free non-bound steroid would explain many of the discrepancies in this area of research. The teleological question of what purpose the "short feedback loop" plays in the intact organism is not yet answerable, and raises the further question, "do such short feedback loops exist?"

The second session covered the effects of ACTH and adrenocortical hormones on the nervous system. All the articles in this section favour the concept that pituitary-adrenocortical hormones exert their influence on the central nervous system at different levels, and that their effects on those structures are also deeply involved in the organization of motivated and conditional behavioural reactions.

The third session covered the effects of ACTH and corticosteroids on animal behaviour. Abundant evidence is presented here that endogenous and exogenous ACTH and adrenal corticosteroids can influence both the acquisition and retention of an active or passive avoidance response. ACTH enhances the acquisition and inhibits the extinction of the response, while corticosteroids induce the reverse. The possibility that active polypeptide fragments of ACTH and MSH have behavioural effects raises the important question whether a number of small behaviourally active peptides are produced by the adreno-hypophysis. The latter part of the book covers the final session on clinical studies of ACTH, corticosteroids and the brain. This section emphasizes the rapid expansion of research in endocrinology and the relationship with the central nervous system. This degree of interrelation-

ship of hormonal and nervous system is so well established that Dr Cleghorn from McGill University points out that this new knowledge must be incorporated in the current knowledge of the informed clinician.

Of particular interest in this section are details that the influence of hormonal excess on growth and development may be to produce a higher IQ, in either the naturally occurring syndrome or those iatrogenically induced by progesterone treatment, in women with threatened abortion. Female issue of such prenatal exposure behave as males. Experiments of this kind could possibly mean pharmacologically induced higher IQ in the future and all its consequences for society. Psychological control of endocrine activity associated with psychotic states is also given in this section.

This book should be read by all neuroendocrinologists, neurophysicists, neurophysiologists and psychiatrists.

DONALD EXLEY

Testis Biochemistry

The Testis. Edited by A. D. Johnson, W. R. Gomes and N. L. Vandemark. Vol. 2: Biochemistry. Pp. xv+468. (Academic: New York and London, September 1970.) £16.35.

THERE has been for some time, and still is, a real need for a good, comprehensive and advanced text on the biochemistry of the testis, a reference book to which the biochemist and physiologist alike could look for information that is now scattered widely in textbooks, review articles and original papers. During the past decade in particular, progress in the biochemistry of testicular tissue has been rapid and impressive, and has expanded knowledge with respect to several groups of chemical compounds. Among those that have received special attention are the enzymes and steroids. As far as testicular enzymes are concerned, the most outstanding achievements include the purification of hyaluronidase and elucidation of its function; some excellent work on nucleolytic enzymes, especially the nucleotidases; the equally important advances concerning the mode of action of several phosphokinases and especially those enzymes which accomplish the phosphorylation of nuclear proteins; and in addition, the separation and identification of various kinds of isozymes. Unfortunately, in *The Testis* hardly any of these accomplishments have received even scant attention, and consequently enzymologists at large will look in vain for answers to the two questions that concern them most—what has been accomplished in the past, and what is worth tackling next?

Biogenic amines and phosphagens, two other important classes of "biochemicals", fare no better than the enzymes. In fact, as regards phosphagens, for example, except for a brief reference to their occurrence in invertebrate testes, there is no mention whatsoever, and I failed to discover in the text a single word about phosphocreatine in the mammalian testis.

The lipids and steroids are dealt with more thoroughly than other groups of testicular constituents. The chapter on lipids is well documented. That on the endocrinology of the testis, however, although rich in information, is rather heavily biased towards selected contributions, and rather strongly coloured by the author's personal idiosyncrasies, as evident from comments on the functional significance of the ratio of androstenedione to testosterone.

On the other hand, the contributions on the nucleic acids in the testis, and the *in vitro* growth and development of mammalian testis, are exceptionally useful. The latter chapter will, no doubt, be of great help to those who may have been wondering whether the gametogenic events of the living testis can really be reproduced in artificial conditions of culture *in vitro*. But many a biochemist will at the same time wonder why this particular chapter, which is largely concerned with problems of organ culture, should have been squeezed into a volume specifically devoted to biochemistry. One might perhaps also ask editors why a group of substances should be referred to in one chapter as DPN and TPN, and in another as NAD and NADP. And why doesn't the subject index include at least one single entry on biochemically important substances such as the cytochromes? T. MANN

Underwater Blood

Circulation in Fishes. By G. H. Satchell. (Cambridge Monographs in Experimental Biology, No. 18.) Pp. x+131. (Cambridge University: London, February 1971.) £2; \$7.

IN his preface to this latest addition to the Cambridge Monographs in Experimental Biology, Professor Satchell states that his purpose has been to attempt to integrate into a coherent account some of the information contained in the several thousand papers dealing with the structure and function of the vascular system of fishes. This difficult task has been well done, and the resulting monograph of 131 pages, which is a pleasure to read, can be warmly recommended. He cites 310 references, selected from material published between 1806 and 1970, but emphasizes that forty per cent of the papers given as references were published in or after 1965.

The subject matter is treated under eleven headings, including the heart, the arteries, the veins, the capillaries, the blood, the control of the heart and the response to hypoxia. A great deal of attention must have been given to what is a skilful grouping and arranging of material and undoubtedly this will help in making the book acceptable to the wide range of readers anticipated (from students in their last years at school to mammalian cardiovascular physiologists, who will find some interesting contrasts in many of the responses described). The text is supplemented by simple, clear figures and drawings.

The short, final chapter on future developments emphasizes the importance of the new "sophisticated" recording and measuring techniques which are becoming available to comparative physiologists (although "sophisticated" would seem to be a wrong description of these advanced techniques). One that is particularly worth mentioning is the use of telemetric recording of physiological information from free swimming fish in their natural environment. Professor Satchell also emphasizes the importance of information provided by the comparative physiologist for the fish cultivation industry, comparable with that available to the other meat-producing industries. It is as well that the pace of this type of research is increasing; there is much leeway to make up if the agricultural and fish cultivation industries are to be served equally in this respect.

F. G. T. HOLLIDAY

Red Cell Membrane

Red Cell Membrane: Structure and Function. Edited by G. A. Jamieson and Tibor J. Greenwalt. (The American National Red Cross Second Annual Scientific Symposium, Washington, DC, May 1969.) Pp. 384. (Lippincott: 1969. Distributed in UK by Blackwell: Oxford.) £8.25.

BOUND in an appropriate bright red cover, this book contains the proceedings of a symposium sponsored by the American National Red Cross in May 1969. Although available for about a year in the United States, it has only just now reached Europe. As might be expected, the symposium covered almost every aspect bearing on the red cell membrane and in that sense was comprehensive. However, some of the authors have concentrated on their own research, while others have attempted to provide a review of their area. Interspersed between the fourteen chapters are six discussions—these are printed much as they must have occurred—which provide some entertainment for the reader and, at times, help him to

see in what the participants were interested and about what they were sceptical.

The first two chapters look at red cells or ghosts through the electron microscope. In the first, use is made of an ion etching technique which produces rather pretty pictures of sponge-like cells, the detail of which is likely to be artefactual rather than biologically significant. The second chapter shows and discusses several pictures of freeze-fractured and etched cells or ghosts together with what we now know is a mistaken interpretation of them. This makes the understanding of text and pictures a little difficult for the reader.

The next section, comprising the chemistry of the membrane, contains several good chapters. This starts with a short survey by Hanahan of some methods for fractionating membrane proteins. This line is pursued by Rosenberg and Guidotti as they describe their original approach to the separation and characterization of different proteins in the membrane. Marchesi and Steers and their colleagues discuss spectrin—that curious but fascinating protein which can be removed from ghosts by chelating agents and which can, in the presence of the right cations, form polymeric filaments. These are located on the inner surface of the cell membrane and are presumably a type of scaffolding to hold the cell in its characteristic shape.

Most of the cell's carbohydrate is found on the outer surface of the membrane, attached either as glycoprotein or glycolipid. The immunology of red cells—covered by Poulik and Bron—is intimately linked with the chemistry of these glyco-compounds. There seems to be just one major glycoprotein in the red cell membrane. As Winzler reported, it contains a hydrophobic polypeptide segment at one end which probably anchors the remainder of the glycoprotein in the membrane. This glycoprotein carries the MN cell antigens and is a most exciting component of the red cell. Most of the remainder of the cell surface carbohydrate is in the form of glycolipids, extensively covered by Sweeley and Dawson. This is a particularly valuable contribution to the book: it contains much of the chemistry known of these compounds (such as eicosasphing-4-ene—hard for all but the chemist), which are so likely to be of profound biological importance. As the authors note at the end, the three-dimensional structures of these compounds should be determined.

The rest of the book is about transport of molecules across the membrane and how the state of the membrane affects the physiology of the cell. This starts with a short chapter on the diffusion of gases through the membrane, followed by a lengthy section on the kinetics of monosaccharide transport by

Miller, presented with a rather historical perspective. A section on the high and low potassium types of sheep erythrocyte—but without any protein chemistry—by Toteson, concludes transport. These chapters are reflected by a Forsythian remark the chairman of the session is recorded as having uttered: "Everything is more complicated than it used to be". The last three chapters include one by Weed and Lacelle on the effects of ATP depletion on red cell physiology.

As is obvious by reading this book, there is a mine of information about the red cell membrane: much more chemical knowledge will, no doubt, be acquired in the next few years. But the real problems are going to be in understanding how all the components in the jigsaw fit together to produce a membrane and then, on a larger scale, how a cell is endowed with such a well defined shape. This poses two immediate problems: how to relate chemically defined components to objects seen in the electron microscope and what is the mechanism by which enzymes involved in transport actually work.

This book is quite well produced, although very few of the electron microscope pictures have a scale on them: this can be very irritating for the reader. There is no author index—which again can be useful. A gratuitous picture appears on page 155 which reappears in its correct place on page 368. But taken all together, as one must do, this book is certainly worth having around for anyone interested in red cells or in membranes generally. The American National Red Cross should be congratulated on having organized such a meeting in the first place. MARK S. BRETSCHER

Development of Viruses

Atlas of the Anatomy and Ontogenesis of Human and Animal Viruses. By A. A. Avakyan and A. F. Bykovsky. Pp. 272+431 electron micrographs and diagrams. (Medicine: Moscow, 1970.) 2.75 rouble.

THIS book, which introduces new concepts of the ontogenesis of viruses, has been written with great scientific passion. The authors have followed their own way by investigating the intracellular development of viruses instead of being interested only in the final forms.

The atlas is richly illustrated (with more than 400 electron micrographs) and summarizes the results of many years of investigation on the submicroscopic anatomy and ontogenesis of human and animal viruses. The presentation revolves around current views on the composition and origin of viruses, structure of virions, penetration of viruses into host cells, intracellular de-

velopment and the release of mature virions from infected cells.

The book consists of an introduction, eleven chapters on the principal groups of animal viruses, a concluding chapter and a bibliography. In each chapter there is a schematic representation of the development cycle of the virus group concerned, with the exception of the first chapter which contains a historical outline of virology and general principles of virus organization.

The literature still lacks up to date interpretation of the virus growth cycle because, instead of detailed analysis of the cycle, most investigators describe only individual phases in the relationship of the virus to the host cell (for example, adsorption, penetration, replication of viral components and their assembly, formation of virions and their release). By present scientific standards this approach is outdated, for the phases in the ontogenesis of a virus ought to be considered separately from the relationship of the virus to the host cell. At present, even people who do not fully agree with the concept of viral ontogenesis accept that viruses pass through a complicated developmental cycle, with the virion representing its final stage.

Morphological, biochemical and physio-functional analysis of the viral ontogenesis enabled Avakyan and Bykovsky a fuller characterization of the vegetative and "sporoidal" stages. Their hypothesis agrees, in principle, with the accepted view that in the developmental cycle of viruses three basic stages correspond to three forms of virus existence—vironucleon, viroplasm and virosporon (this last term should be considered analogous to the "sporophyte" of Penso, 1955). They recognize, however, the existence of some additional stages. Accordingly, on entering a cell the virionucleon induces the formation of a "polygenomic" viroplasm which is associated with systems required for nucleic acid replication, synthesis of specific viral antigens and the assembling of these compounds. At this stage intensive processes of interchanging, multiplication and growth take place which morphologically present themselves as an increase of mass, volume and complicated structural organization. The obligatory phase in differentiation of "polygenomic" viroplasm is the formation of "monogenomic" viroplasm which in itself is the initial ontogenetic stage of the new virus generation.

According to the authors the complete developmental cycle of a virus consists of five stages reflected in five forms: "monogenomic" viroplasm, provirion, virion, "viropartus" and virionucleon. The "monogenomic" viroplasm consists of viral genome, informational RNA, a complex of specific viral enzymes and structural proteins. This stage terminates

with the *de novo* formation of the viral membrane. The provirion is the next stage in which the virus, on completion of its external membrane, separates from the viroplasm and undergoes differentiation of its structures. Gradually the dynamic processes become less intensive, differentiation and exchange come to a halt and this leads to the formation of a virion. This form, enclosed within a nucleocapsid, protects and safeguards the viral genome and morphology. There is a direct relationship between the degree of differentiation of structures in the provirion and of the virion's organization and the level of evolutionary development of each virus. The next stage is "viropartus", a form enabling the release ("delivery") of the viral genome from the nucleocapsid and its penetration into the host cell. The "delivery" of viral genome from the virion has not yet been elucidated but in the case of many bacterial viruses there is a special mechanism for this purpose.

Vironucleon, the "germinating" form, is the released ("delivered") viral genome which functions by passing the virus-specific genetic code to a suitable host cell and by induction of the formation of a "polygenome", thus completing the growth cycle.

The first two stages belong to the vegetative phase of the viral developmental cycle, virion and viropartus to the "sporoidal" phase and virionucleon represents the "seed" which conveys the genetic message specific for the virus.

Avakyan and Bykovsky trace a direct relationship between the degree of complexity in viruses organization and the complexity of their ontogenetic developmental cycle: the more complicated the anatomy of the virion, the longer the differentiation process the virus passes through.

The virological literature has not had, so far, an atlas of such quality, richness of content and wealth of thought. It would be of great value to scientists who do not know Russian if it were made accessible by translation into a western language, preferably English.

J. W. CZEKALOWSKI

Biochemistry Takes Stock

British Biochemistry—Past and Present. Organized and edited by T. W. Goodwin. Pp x+228. (Academic: London and New York, November 1970.) £3.00; \$9.

THIS book records the occasion of the 500th meeting of the Biochemical Society in December 1969, when many distinguished biochemists held a symposium to discuss the history and present state of various aspects of biochemistry. The chief headings and speakers were

molecular biology (Davidson, Kendrew, Phillis, Hartley, Pirie); immunology (Marrack, Humphrey, Porter, Morgan); intermediary metabolism (Neuberger, Krebs, Popjak, Kornberg), and separation methods (Martin, Synge, Sanger, Brownlee, James). The balance between past and present varies according to the taste of the author but in general the balance is good. The views presented are not too parochial and it is stressed in most of the articles that the study of biochemistry is international and that the reactions transcend most international energy barriers. The book should perhaps be read in conjunction with *The Biochemical Society, its History and Activities 1911-1969* by R. A. Morton, published by the Biochemical Society, to give an additional view of the development of the study of biochemistry in Britain.

The flavour of the present book can be illustrated by a few quotations. Can you guess who wrote them?

"I was convinced at the time that studying intermediary metabolism in an academically orientated hospital was justified because I felt that sooner or later any information on intermediary metabolism would be relevant to clinical problems."

"One final year examination paper in biochemistry in 1929 was, as usual, headed by some such words as 'candidates should not attempt more than three questions'. One answer seemed therefore to be permitted and I spent the whole period on nucleic acids. I was reproved but passed the examination."

"Nostalgia is an emotion which is invariably pleasant for a speaker, occasionally appreciated by an audience, but seldom tolerable in print."

"My own first sight of a chromatogram was in the biochemical laboratory in 1937 or thereabouts. It was a very beautiful one, which had been made by the late Ernest Baldwin. He had made it from an extract of sea urchin pigments."

"How could I convey now at a distance of more than 20 years the joy I experienced when the lights on my first scaler started to flicker on bringing under the Geiger counter the first sample of ^{32}P ? The pleasure was as great as when the lights went up again on Westminster Bridge after years of darkness."

"Crystals, for all their beauty, seem essentially dead and the least likely objects of study by biochemists but they have proved most important to the development of biochemistry on more than one occasion."

"He was clearly a remarkable man in many ways as I have been told that he proceeded directly from Stockholm to Monte Carlo where by judicious play he doubled the prize money and then withdrew."

"Today—and this is a change which has occurred recently — progress in science often depends decisively on new ideas and new theoretical concepts, whilst in the past methodology was perhaps the most important single limiting factor. Powerful tools such as those provided by isotopes, by optical methods of analysis and by automation have made available a very effective technology. Now we have the tools and the means of getting better tools but we do not always know what to do with them, because of the lack of good ideas."

If you cannot guess the authors, then this information and other data of value and interest will be yours after you have read the book. G. A. KERKUT

Electronic Eyes

Photoelectronic Imaging Devices, Vol. 1: Physical Processes and Methods of Analysis. Edited by Lucien M. Biberman and Sol Nudelman. (Optical Physics and Engineering.) (Plenum: New York and London, 1971.)

THE dark-adapted human eye is capable of detecting a pulse of less than a hundred photons. The need for electronic devices which may be used to increase this surprisingly large efficiency may not be apparent until it is realized that it is not particularly convenient to use the eye as a detector for such small signals; the process of dark adaptation is lengthy, and high detection efficiencies are only obtained in the green region of the spectrum. There is a rapidly growing market (at scientific, commercial and military levels) for devices which will allow the detection and viewing of signals consisting of relatively small numbers of photons in parts of the spectrum as widely separated as the infrared and X-ray regions. Another very important type of instrument is the signal-generating tube, as used in commercial television cameras; here an optical signal is converted into an electrical output.

The principles underlying the operation of these devices are to be found in solid state physics (photoelectric emission, secondary electron emission, photoconductivity and luminescence), optics, electron optics and information theory. University studies in this field have been established in Britain for many years; the applied physics section of the Physics Department at Imperial College, London, has an international reputation. An idea of the way in which the subject has grown may be obtained from a study of the proceedings of the four international symposia on photoelectronic image devices which have been held at Imperial College at regular intervals since 1958.

The University of Rhode Island has recently introduced the first full scale postgraduate course on photoelectronics in the United States and this book, with its companion volume, arose out of a series of lectures delivered at that university by American and British experts, and it has since been adopted as the basic textbook for the course. The first volume covers the basic physical and mathematical principles of photoelectric imaging devices, ranging from sections on photometric quantities and the visual detection process to the relevant parts of solid state physics and electron optics. Methods of analysis and specification of the performance of image tubes are discussed, and there is also a section on system evaluation.

If a book such as this is presented as the proceedings of a symposium or a summer school, the reader may expect a variation in the depth of treatment used by the various contributors. On the other hand, if it is intended to be a textbook, he is entitled to assume that a considerable degree of editing has been carried out so as to obtain a homogeneous standard. The level of treatment in this book varies widely, as can be illustrated by considering neighbouring chapters on photoconductivity and photoelectric emission. The presentation of a simple theoretical model of photoconductivity is an excellent summary and extension of material that can be obtained in basic solid state texts; the chapters on photoemission deal almost entirely with descriptions of the preparation and properties of selected photocathode materials, and the basic physical processes are somewhat neglected. A detailed criticism of the chapter on recent photocathode developments is that the information presented on the alkali antimonides goes no further than the early 1960s; there is no mention of the very efficient K_2CsSb cathode, which was discovered by Sommer in 1964.

The lack of thorough editing shows up in a rather amusing way. Biberman's chapter on photometric quantities is excellent, and fully bears out his stated intention to "say what he means, and mean what he says". The next chapter, provided by the same contributor, includes a discussion of the irradiance through various optical air masses; the concept of a unit air mass will be unfamiliar to many readers, yet nowhere is it defined. Similar criticisms apply to the chapter on photoemissive materials. The alkali antimonides are referred to by the S-number notation, which is not explained in this volume; because of its arbitrary nature a newcomer to the subject may find this nomenclature very confusing. There are a number of trivial but annoying spelling and printing errors, and there is a lack of uniformity in the presentation of the dia-

grams. In many cases these have been reduced to such a small size that the labelling of the axes is almost illegible. There is no name index and the bibliographies provided at the end of each chapter are seldom exhaustive.

Some parts of the book are excellent and will be of considerable value to workers outside this field. I found Biberman's chapter on luminance, radiance and temperature, and Vine's contribution on electron optics, particularly good in this general sense. For specialists many other chapters will be very welcome, but the publishers' claims for the book's recognition as an encyclopaedic reference work and as the basic textbook in the field must be treated with caution.

C. H. B. MEE

Semiconductors

Organic Semiconductors and Biopolymers. By L. I. Boguslavskii and A. V. Vannikov. Translated from the Russian by B. J. Hazzard. (Monographs in Semiconductor Physics, Vol. 6.) Pp. xii+221. (Plenum: New York and London, 1970.) \$18.50.

THIS monograph has been produced with the intention of providing a stimulus to physicists and solid state chemists outside the Soviet Union who are working on organic semiconductors and, more specifically, conjugated, polymeric, organic semiconductors. The reader is assumed to be familiar with not only the background theory but also the chemical nomenclature; but the text is so lucid that it could be of considerable interest to the informed layman. Unfortunately, a delay of two years in translating the book from the Russian, coupled with the fact that the literature is only covered adequately up to the beginning of 1967, means that the book is now of very limited value to active research workers in a field which is expanding so rapidly.

The authors begin by outlining briefly the synthetic background to the field, ignoring the many problems associated with chemical characterization which have deterred others. Much of the text is somewhat uncritical; for example, in a brief discussion of the compensation effect they make no reference to the controversy still surrounding this topic.

There is an adequate review of the photoconducting properties of these compounds but little is said about the theoretical advances or experimental techniques.

The authors then discuss the connexion between the electrical and paramagnetic characteristics of polymeric semiconductors emphasizing the difficulty of drawing well founded conclusions about the mechanisms of the electronic phenomena observed because

of the difficulties of determining unambiguous structures. They recognize the part that electron paramagnetic resonance spectra can play where there is a connexion between the paramagnetic and electro-physical properties.

This leads into a discussion of the mechanisms of conduction, which is the major part of the work; it is also the field in which both authors have been actively engaged. They begin with brief, critical reviews of the Hall, Seebeck, and a.c. conductivity techniques and continue by interpreting the obtained results on the basis of a model which assumes, in many cases, a highly conducting layer separated by thin barriers. There follows an examination of the techniques for measuring directly the mobility of the current carriers, by examining the drift of the excited carriers in an electric field. The authors consider this technique to be very important in view of its applicability to single crystals, polycrystalline layers of low molecular weight, and thin polymeric films.

In a field where, in the past, contradictory results have been obtained by the confusion of bulk and surface effects, there is a valuable review of surface properties. Particular stress is laid on the effects of adsorbed gases on the bulk properties. This leads logically to an interesting discussion pointing out the connexion between paramagnetic centres and catalytic activity.

Although the title of the book includes the word "biopolymers" these compounds are covered in a surprisingly brief chapter; although unlikely to be of great use to workers active in the field, it may usefully stimulate others to take an interest in biological systems.

The book concludes with an interesting discussion of the practical applications of this work.

R. G. EVANS

Field Effect Transistors

Field Effect Electronics. By W. Gosling, W. G. Townsend and J. Watson. Pp. xvii+364. (Butterworth: London, March 1971.) £8.

THE number of field effect electronic devices available for use is increasing, and if the designer is to make the best use of these devices he should understand the principles on which they operate. In this book, the authors have described the physical principles on which field effect devices operate, the characteristics of the devices, and have given some chapters on their uses. The book is intended for final year undergraduate or postgraduate courses or for the practising engineer who wishes to become familiar with potential uses.

The first three chapters are devoted to the historical development and to the

physics of operation of field effect devices of both the junction and insulated gate types. A mathematical description of the operation of the devices is coupled to a description of their physical construction. The physical descriptions illustrate some of the limitations imposed on the construction of devices and also show how arrays of devices may be made and utilized. The manufacturing processes required are described in sufficient detail to give an appreciation of the difficulties in the processes to those who will not be concerned with the actual production of devices.

The remaining twelve chapters are concerned with the operation of field effect devices in circuits. A principal limitation to the use of any electronic device is the noise introduced by the device into the circuit in which it is to operate. A chapter is devoted to this topic, discussing the sources of noise and the noise equivalent circuits. The setting up of equivalent circuits for devices is an important topic both to assist in understanding the operation of devices and for use in the design of circuits, particularly when using computer aided design methods. A chapter is devoted to the characteristics and equivalent circuits of junction and insulated gate devices. Several chapters are given to discussing the use of field effect devices in different types of linear circuits. There are some numerical examples to illustrate the use of design formulae but more might have been included to give a better feeling for the design of practical circuits. There is little comparison between the performance of circuits using field effect devices and those using bipolar transistors. This is surely an important consideration to be taken into account by the circuit designer who will wish to know when it is of advantage to use field effect devices in preference to bipolar devices. Two chapters are devoted to a discussion of integrated circuit devices, an important and rapidly developing field of use for field effect devices, particularly in the production of large scale integrated logic circuits.

This book gives a clear presentation of the physical operation and use of field effect devices. The level at which it is written should present little difficulty to final year undergraduates; indeed the level is such that it might be used earlier in an undergraduate course using the chapters on circuit design as an introduction to the various types of circuits discussed, in parallel with a discussion of the corresponding bipolar transistor circuits. The practising engineer who is used to bipolar transistor design will find this book useful to refer to as an introduction to the use and limitations of field effect devices.

P. A. MATTHEWS

CORRESPONDENCE

Lawrence Memorial Fund

SIR,—We should be grateful for space in your columns to draw attention to the establishment of a fund to institute a memorial to Professor A. S. C. Lawrence (for obituary see this issue of *Nature*, page 70).

Professor Lawrence worked in this department for the past twenty-four years and both his recent colleagues and others whom we have consulted feel that it would be appropriate if the memorial were specifically associated with the University of Sheffield, and, in particular, the Department of Chemistry. Accordingly we hope to set up a fund to provide an award for meritorious work in chemistry by an undergraduate in this department. We hope to contact personally as many as possible of Professor Lawrence's former colleagues and friends but the list of these is very long and it seems likely that we shall miss some. We should be very grateful if people whom we fail to contact but who are interested in contributing would contact one of us for further information.

Yours faithfully,

N. M. ATHERTON
B. BROCKLEHURST
K. R. JENNINGS

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ESRO On-line

SIR,—In your leading article devoted mainly to the question of the future of the European Space Research Organization (ESRO), you say that ESRO should aim to support, among other things, "more down to earth activities... such as... the collecting and cataloguing of information (still sorely neglected in the madcap race for information)" (*Nature*, 231, 273; 1971). As the officer responsible for the ESRO/ELDO Space Documentation Service, I feel that I must point out that we are already operating one of the foremost and most comprehensive documentation systems, in Europe, if not in the world.

This service is based on the remote console (RECON) interrogation system developed for NASA, and depends on a generous exchange agreement with NASA,

which must be given its true value in terms of "winning benefits worth having from the application of space research". In fact the RECON system itself is a prime example of "fall-out" from such research. However, ESRO has not been content with offering the benefits of its advanced documentation service to its own staff. This has now been made available directly to national centres, and consequently industry, through the medium of remote on-line terminals. The system was recently demonstrated at the Society of British Aircraft Constructors, and the installation of a terminal in the UK Technology Reports Centre announced in the press.

Although, as you say in your article, ESRO made in its early days a slow start, I think that this situation has clearly changed, and once again the documentation service itself proves this point, inasmuch as it is now providing an even more comprehensive information base to its users by including not only the half million documents of the NASA file, but almost another half million provided by reports in the metallurgical field (Metals Abstracts), engineering (Engineering Index), and by reports generated in the US Government Research and Development programme. Other files in the chemical field will soon follow.

To my knowledge there is no other on-line information system available, offering such a rich source of knowledge, while at the same time combining the economies of centralized file processing for many users, together with the efficiency of local retrieval operations.

Yours faithfully,

N. E. C. ISOTTA

*Documentation and
Library Service,
ESRO,
Neuilly-sur-Seine*

Aid for Bengal

SIR,—You have very rightly reproached the developed nations for the sad condition of Bengal (*Nature*, 231, 341; 1971). But even more deplorable is the complete lack of understanding for the urgency of the problem by the bureaucratic machinery of the government of developing nations. Press and television have reported that even after two weeks

or more the aid received from abroad is still blocked in governmental channels. I agree that the supplies of cholera vaccines and saline solutions were belatedly flown from abroad into India but the bureaucrats in the machinery of Indian government itself are further delaying their delivery to the institutions and hospitals urgently requiring them. It is inexcusable that the bureaucratic machinery of the Indian government itself showed no humane concern for the people for whom the world outside India, especially the United Kingdom, is so concerned.

I certainly support, wholeheartedly, your idea of an international organization such as United Nations, or one of its agencies, being given a lien on worldwide facilities to deal with international disasters, whether they be nature or man made. The present evidence is, however, that the United Nations Refugee Organization has done practically nothing in the case of the Bengal disaster. It would appear to be due to lack of dedicated leadership. Private charity organizations such as Oxfam, War on Want and Christian Aid from abroad and the local relief organizations, similar to the one led by Mother Teresa, on the other hand, have achieved more success in spite of their meagre resources, due solely to their sincere and dedicated leadership.

Yours faithfully,

K. C. TRIPATHI

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Caveat Lector

SIR,—Whatever their individual feelings may be about changes in the language, I imagine that all scientists disapprove of the introduction of ambiguity where formerly there was none, and furthermore that in most cases there would be general support for the removal of traditional ambiguities, unless these were useful.

Both kinds of ambiguity are illustrated to your disadvantage in *Nature* of June 18, 1971. The first often results from using (adjective + noun) as (noun) and is now widely disfiguring technical literature in the English language. As one example among many in that issue of *Nature*, on p. 410, second paragraph,

we find reference to the "six year old recommendations of the Littlewood committee". A moment's thought suffices to reject the possibility in this context of six recommendations, each a year old. Yet why must we be constantly brought up against these small ambiguities, all of which would vanish on the appearance of correctly placed hyphens? In this case the two meanings are rendered by "six-year-old" and "six year-old".

A traditional ambiguity is to be found in the title on p. 432: "Sex Determination in Birds and Mammals". The *Concise Oxford Dictionary* actually lists the two conflicting meanings of "determine" consecutively: "give decision; be the decisive factor in regard to". Inspection of the article showed me that I had taken the wrong meaning in supposing the authors to have new ideas on finding out whether individual birds are male or female. Therefore a better title might have been "Sex Regulation. . .", whatever the customary usage among biologists may be.

Of these two kinds of ambiguity the first is by far the most pervasive and deleterious. *Nature* is difficult enough

to understand without these unnecessary obstacles.

Yours faithfully,

GARRY THOMSON

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London SW1*

Braille Reading

SIR,—Perhaps I may be permitted to make two points on the report by Hermelin and O'Connor on braille reading (*Nature*, 231, 470; 1971).

In a survey I made three years ago on methods of teaching braille to newly blinded adults and adolescents, one of the questions asked teachers to state what advice they gave learners as to which hand to use in reading. Two of the 67 respondents stated that they advised the use of the right hand alone; none recommended the left hand alone; 29 recommended the use of both hands; 36 allowed the learner to make his own decision.

The justification for the "scissors" movement described by Hermelin and

O'Connor is that while the right hand is completing the second half of a line, the left hand can be dropped down to the next line, thus ensuring a quicker rate of reading. As only about 30% of all braille readers read at more than 100 words per minute, it is clearly desirable to develop the use of both hands so that as little time as possible is lost in moving from line to line. One of the most readily observable characteristics that differentiates the fast brailist from the slow is the extent to which the former uses both hands. To take advantage of the Hermelin and O'Connor findings we should probably need to read in a right to left direction so that the good hand, the left, can do the reading while the less efficient right drops down to the next line to act as a marker for the returning left!

Yours faithfully,

M. J. TOBIN

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Obituary

Professor S. Lawrence



STUART LAWRENCE was born in London on June 28, 1902, and died in Sheffield on February 22, 1971. His early education at Christ's Hospital School was continued with evening classes at Battersea Polytechnic, but the moulding of the essential personality of Lawrence the scientist really began when Sir James Dewar recruited him as an assistant at the Royal Institution, where he remained until 1929. During that time he rubbed shoulders

with the famous, witnessed an important phase in the development of X-ray analysis, and acquired a useful familiarity with the standard techniques of a physical science laboratory. In 1929 he moved to Cambridge. While a lively interest in photography produced *The Scientific Photographer* (1941), it was his other early book on *Soap Films* (1929) which showed where his heart really lay; he was already an experienced investigator in that field at the time of his move to the Colloid Science Laboratory at Cambridge, on a senior D.S.I.R. award. At Cambridge he proceeded directly to the Ph.D. degree and subsequently to the Sc.D. His F.R.I.C. dates from 1944.

During the war, scientific advisory work for the Admiralty took him to sea to examine at first hand the problems of de-icing and seawater emulsions in fuel oils and sent him finally, with the rank of commander, on a scientific tour of devastated Europe. That work led to two by-products: a Beilby award in 1948 and a fund of sea stories with which to lace his general conversation.

He joined the Chemistry Department of the University of Sheffield as a Senior Lecturer in 1947, but his distinction as a researcher was recognized by the award of a readership in 1949 and of a personal

chair in 1966, a year before his retirement. He will be remembered with great affection by his colleagues, his research collaborators, and by many generations of undergraduates for his was a personality that could not be overlooked: it had an endearingly comic streak that was perhaps unsuspected from a chance professional encounter with the great domed head and the mock-pompous expression he reserved for special occasions. His tremendous reserves of courage were shown in his attitude towards the amputation of a leg in 1964: within four or five months he was already flying to foreign parts to fulfil his lecturing and other commitments.

Though he initiated quantitative investigations from time to time—for example, in his studies of viscosity with A. Jobling and of coagulation with O. S. Mills—his great strength lay in the intuitive feeling he had for the physical nature of colloidal and liquid crystal systems. He was happiest amongst thermostats, test-tubes and microscope slides, and his enthusiasm for the polarizing microscope and the myelinic figures he saw with it often suggested the thought that, if he had not been a distinguished colloid scientist, he might well have become an outstanding descriptive biologist.

Announcements

University News

Professor John D. Isaacs, professor of oceanography at the Scripps Institution of Oceanography, has been appointed director of the Institute of Marine Resources, University of California.

Professor John T. Tate has been appointed Perkins professor of mathematics at Harvard University in succession to **Professor Richard D. Brauer** and **Professor G. M. A. Hanfmann** has been appointed John E. Hudson professor of archaeology in succession to **Professor Sterling Dow**.

Dr P. G. Harper, University of Reading, has been appointed to the new chair of theoretical physics at Heriot-Watt University.

The following appointments to chairs have been made in the University of London: **Dr Ian Butterworth**, to the chair of physics, Imperial College; **Professor J. M. Ledingham**, to the chair of medicine at the London Hospital Medical College; **Dr M. H. Lessof**, to the chair of medicine at Guy's Hospital Medical School; **Mr I. McColl**, to the chair of surgery at Guy's Hospital Medical School; **Professor J. K. T. L. Nash**, to the chair of civil engineering at King's College; **Dr P. J. Peterson**, to the chair of botany at Westfield College; **Dr A. S. Truswell**, to the chair of nutrition and dietetics at Queen Elizabeth College; **Dr C. W. Turner**, to the William Siemens chair of electrical engineering at King's College. The title of professor of oral immunology has been conferred on **Dr T. Lehner**, in respect of his post at Guy's Hospital Medical School.

Dr David S. Campbell, Plessey Company Limited, has been appointed to a chair in electrical engineering at Loughborough University of Technology.

Mr H. B. Rodgers, University of Keele, has been appointed to a chair of geography in the University of Manchester, and **Miss V. R. Cane**, University of Cambridge, has been appointed to the chair of mathematical statistics. **Mr W. R. Lee** will succeed **Professor T. S. Scott** as professor of occupational health.

Dr Bruce J. H. McKellar, University of Sydney, has been appointed to the chair of theoretical physics in the University of Melbourne, in succession to **Professor C. B. O. Mohr**, and **Dr H. H. Bolotin**, Argonne National Laboratory, has been appointed to the Chamber of Manufacturers' chair of physics.

Professor F. H. T. Rhodes has been appointed dean of the College of Literature, Science and the Arts at the University of Michigan.

Mr M. W. Barley has been appointed professor of archaeology and **Dr James Crossland** has been appointed professor of pharmacology, both in the University of Nottingham.

Dr D. G. Grahame-Smith, St Mary's Hospital Medical School, has been appointed professor of clinical pharmacology in the University of Oxford and honorary director of the MRC Clinical Pharmacology Unit to be established there.

Dr R. Wright, University of Oxford, has been appointed to the second chair of medicine in the University of Southampton. **Mr H. J. Trump** is retiring as director of extra-mural studies, and will be succeeded by **Mr P. E. Fordham**.

Appointments

Mr F. J. Doggett and **Mr A. M. Allen** have been appointed full-time members of the United Kingdom Atomic Energy Authority.

Mr E. C. Hulse, head of the Biological Products and Standards Department of the Central Veterinary Laboratory, Weybridge, has been appointed a deputy director of the Laboratory.

Mr Michael Armstrong, coordinator of the Cargo Handling Department of Vickers Shipbuilding Group, has been appointed secretary general of the Association of British Oceanological Industries.

Dr Andrew Davis, until recently project leader and director of the WHO/MRC/Government of Tanzania Bilharziasis Chemotherapy Centre at Tanga, Tanzania, has been appointed director of the MRC Epidemiology Unit in Jamaica.

Miscellaneous

The Meldola medal for 1970 has been awarded by the Council of the Royal Institute of Chemistry to **Dr G. M. Sheldrick**, University of Cambridge.

Dr S. W. Hawking, Institute of Theoretical Astronomy, Cambridge, has been awarded the 1971 prize of the Gravity Research Fund, New Boston.

The Clarke Institute of Psychiatry Research Fund prize for 1971 has been awarded to **Dr A. A. Boulton**, University of Saskatchewan, for his research on the non-catecholic primary aromatic amines implicated in various neurological and psychiatric disorders.

The trustees of the Lady Tata Memorial Trust for research in leukaemia have made the following awards for the academic year 1971-72: **G. B. Rossi** (Rome), fellowship in second year; **J. F. Doré** (Paris), **T. J. E. Rytomaa** (Helsinki) and **B. Szafarz** (Paris), expenses grants renewed; **T. M. Dexter** (Manchester), whole time scholarship renewed.

At the 1971 annual general meeting of the Society of Cosmetic Chemists of Great Britain, **Mr J. S. Cannell** was elected president of the society for 1971-72. Vice-president will be **Mrs Hilda Butler**, honorary secretary **Mr J. C. McCarthy**, and honorary treasurer **Mr G. A. C. Pitt**. **Mr B. Burgess**, **Mr D. E. Butteraeld** and **Miss A. E. Young** were elected council members.

Mr A. Fraser McIntosh, Rentokil Group Ltd, has been elected president of the British Pest Control Association; vice-president is **Dr A. G. Fiskin**, Shellstar Limited, and honorary treasurer **Mr F. V. Tiffin**, H. Tiffin & Son Ltd. Members of the executive committee include **Mr W. H. Booth**, **Mr J. David**, **Mr S. P. Egleton**, **Mr A. L. Hopper**, **Mr W. A. Potter** and **Mr D. M. Simpson**, who was also elected honorary vice-president of the Association.

International Meetings

July 2, **History of Mathematics**, London (Dr J. M. Dubbey, Department of Mathematics, Thames Polytechnic, Wellington Street, London SE18 6PF).

July 6-8, **Electronic Control of Mechanical Handling**, Nottingham (Conference Department, Institution of Electronic and Radio Engineers, 9 Bedford Square, London WC1B 3RG).

July 7-14, **Environmental Problems**, Istanbul (Halis Odabasi, Joint Institute for Laboratory Astrophysics, University of Colorado, Boulder, Colorado 80302, USA).

July 8-9, **Trends in Chemical Education**, Sheffield (Dr R. J. D. Rutherford, Sheffield Polytechnic, Department of Chemistry, Pond Street, Sheffield S1 1WB).

July 8-9, **Biochemical Society Meeting**, Oxford (Executive Secretary, The Biochemical Society, 7 Warwick Court, High Holborn, London WC1R 5DP).

July 9-11, **Problems in Communication**, Cambridge (Mrs Kate MacSorley, 238 Camden Road, London NW1).

July 18-23, **Fluorine Chemistry**, Durham (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

July 31-August 7, **Conferences of the Institute of Medical Laboratory Technology**, Aberdeen (A. D. Farr, Regional Transfusion Centre, Royal Infirmary, Foresterhill, Aberdeen AB9 2ZW).

August 25-27, **Canadian High Polymer Forum**, Waterloo, Ontario (Dr D. J. Worsfold, National Research Council of Canada, Ottawa K1A 0R9, Canada).

August 29-September 3, **Information Science**, Tel Aviv (The Organizing Committee, International Conference on Information Science, PO Box 16271, Tel Aviv, Israel).

August 30–September 2, **Tritium**, Las Vegas (Tritium Symposium Committee, Southwestern Radiological Health Laboratory, PO Box 15027, Las Vegas, Nevada 89114, USA).

Errata

In the article "Evidence that Piroplasms can undergo Antigenic Variation" by R. S. Phillips (*Nature*, **231**, 323; 1971), the key to the symbols in Fig. 1 should read: ●, Immunized; ▲, controls.

In the article "Solvophobic Interactions and Micelle Formation in Structure Forming Nonaqueous Solvents" by Ashoka Ray (*Nature*, **231**, 313; 1971), the sentence beginning five lines beneath equation (1) on p. 313 should read: "Such ΔG_m values should therefore be taken as only approximate." Because of an editorial error, an unrevised version of Table 1 was published. The correct version appears here.

Notes to Authors

Will contributors to *Nature* follow as far as possible the general style of the journal, and in particular the following rules:

1. Manuscripts should be typed in double spacing and submitted in duplicate. Two copies of all figures should be included, one for the printers and one for the referee.

2. References are indicated by superscript numbers in the order in which they appear in the text, and have the following form:

¹ Sargent, W. L. W., and Searle, L., *Astrophys. J. Lett.*, **162**, L155 (1970).

² Fraenkel-Conrat, H., in *Comprehensive Biochemistry* (edit. by Florkin, M., and Stotz, E. H.), **7**, 75 (Elsevier, Amsterdam, 1963).

"Unpublished work", "Private communication" and "Work in preparation" should not be cited as references but may be incorporated in the text. Each reference number should refer to an individual work which has either been published or is in the press.

3. Although the text of longer articles is usually interrupted by headings at suitable intervals, these should be short and informative and not merely "Introduction", "Results", or "Conclusion".

4. Units should either conform to the SI system or be spelled out in full.

A fuller guide can be had from the *Nature* offices in London and Washington.

Table 1 Observed Solvophobic Effects and Bulk Properties of the Solvents

Solvent	Dielectric constant	Surface tension (dynes cm ⁻¹)	Solubility parameter (calorie ^{1/2} cm ^{-3/2})	Critical micelle concentration of NPE ₉ at 27.5° C (mol fraction)	– ΔG_m (kcal-ories mol ⁻¹)
Class I					
Water	78.5	72	23.4	1.01×10^{-6}	8.24
Glycerol	42.5	63	15.4	8.01×10^{-6}	7.00
Formamide	109.5	57.6	19.4	6.31×10^{-4}	4.40
Ethylene glycol	37.7	48.9	15.5	7.06×10^{-4}	4.33
2-Aminoethanol	—	50.3	—	1.25×10^{-3}	3.99
1,3-Propanediol	35.0	49.2	13.5	4.72×10^{-3}	3.20
1,4-Butanediol	—	47.4	—	1.55×10^{-2}	2.49
Formic acid	58.5	38.1	—	2.17×10^{-2}	2.29
Ethylenediamine	14.2	42.9	12.0	3.12×10^{-2}	2.07
2-Mercaptoethanol	—	45.7	—	3.89×10^{-2}	1.94
1,3-Butanediol	—	39.1	—	4.60×10^{-2}	1.84
1,2-Propanediol	32.0	38.0	13.3	4.98×10^{-2}	1.79
1-Amino-2-propanol	—	36.5	—	5.50×10^{-2}	1.73
Class II					
N-Methylformamide	182.4	40	16.1	—	—
Dimethylformamide	36.7	37.7	12.2	—	—
Diiodomethane	5.3	50.7	11.8	—	—
Class III					
Methanol	32.6	22.6	17.2	—	—
Ethanol	24.3	22.3	12.9	—	—
Toluene	2.4	28.5	8.9	—	—

Reports and Publications

not included in the Monthly Books Supplement

Great Britain

Potato Marketing Board. Sutton Bridge Experimental Station. Report No. 7: The Harvesting, Handling and Storage of Potatoes in Bulk and Boxes. By R. J. Callis. Pp. ii+9. (London: The Potato Marketing Board, 1971.) [294]

National Physical Laboratory. The Calibration of Thermometers. By C. R. Barber. Pp. 48. (London: HMSO, 1971.) 35p net. [294]

Freshwater Biological Association. Scientific Publication No. 22: A Guide for the Identification of British Aquatic Oligochaeta. By R. O. Brinkhurst. Pp. 55+1 plate. Second edition, revised. (Ambleside, Westmorland: Freshwater Biological Association, 1971.) 30p. [304]

Broadcasting and Society. By Charles Curran. (Speech given at the Edinburgh Broadcasting Conference, March 23, 1971.) Pp. 19. (London: BBC, 1971.) [304]

Chatham House; and PEP. European Series No. 17: The EEC: National Parliaments in Community Decision-making. By Michael Niblock. Pp. 112. (London: Chatham House; and PEP, 1971.) £1. [304]

Department of Trade and Industry. Report on the Nuclear Ship Study. Pp. iii+110. (London: HMSO, 1971.) 70p net. [35]

Publications of the Royal Observatory, Edinburgh, 1971, Vol. 8, No. 11: Automation in Optical Astrophysics. (The Proceedings of Colloquium No. 11 of the International Astronomical Union at Edinburgh, August 12/14, 1970.) Pp. viii+211. (Edinburgh: The Royal Observatory, 1971.) £5 net. [35]

Department of the Environment and Fire Offices' Committee Joint Fire Research Organization. Fire Note No. 12: Expanded Polystyrene Linings for Domestic Buildings. By H. L. Malhotra. Pp. 7. (London: HMSO, 1971.) 40p net. [65]

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